Can Eurasian Watermilfoil Be Managed In Minnesota By Biological Control With Native Or Naturalized Insects?

Final Report submitted as Deliverable 5

BY

Raymond M. Newman[1], David W. Ragsdale[2] & David D. Biesboer[3]
[1]Department of Fisheries & Wildlife
[2]Department of Entomology and
[3]Department of Plant Biology
University of Minnesota
St. Paul, MN 55108

TO

Charles Welling
Eurasian Watermilfoil Program
Ecological Services Section, Box 25
Minnesota Department of Natural Resources
500 Lafayette Rd.
St. Paul, MN 55155-4025

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Introduction

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an exotic aquatic weed that was introduced to North America between the late 1800s and the early 1940s (Aiken et al. 1979, Smith and Barko 1990). It is a major nuisance species in eastern North America, the Pacific Northwest and the upper midwest (Grace and Wetzel 1978, Aiken et al. 1979, Smith and Barko 1990). It was first reported in Minnesota in 1987 and now occurs in over 70 lakes and four streams in Minnesota (Exotics Species Programs 1996). The largest infestations are located in the Twin Cities metropolitan area, including Lake Minnetonka. The ability of *M. spicatum* to displace other aquatic macrophytes (Madsen et al. 1991) and its rapid dispersal by fragmentation and vegetative reproduction enable it to invade waterbodies and attain nuisance levels (Grace and Wetzel 1978, Smith and Barko 1990). The species grows rapidly and tends to form a dense canopy on the water surface, which, along with the submersed growth, often interferes with recreation (Smith and Barko 1990), inhibits water flow, impedes navigation, and may clog water intakes (Grace and Wetzel 1978).

Recently, interest has increased in potential of native and naturalized invertebrates as biological controls of Eurasian watermilfoil. Declines of the plant have been associated the occurrence of populations of three herbivores: the moth *Acentria ephemerella* (Denis & Schiffermüller) (= *Acentria nivea* (Olivier)) a naturalized Pyralidae, the indigenous midge *Cricotopus myriophylli* Oliver and the indigenous weevil *Euhrychiopsis lecontei* (Dietz) (= *Eubrychiopsis lecontei*). All three taxa are present in Minnesota and Wisconsin (Newman and Maher 1995). The caterpillar of *A. ephemerella* has been associated with Eurasian watermilfoil declines in Ontario (Painter and McCabe 1988) and Vermont (Creed and Sheldon 1994a), *C. myriophylli* larvae have been associated with Eurasian watermilfoil declines in British Columbia (Kangasniemi and Oliver 1983, MacRae et al. 1990, MacRae and Ring 1993, Kangasniemi *et al.* 1993), and adults and larvae of *E. lecontei* have been associated with declines of Eurasian watermilfoil in Vermont (Creed and Sheldon 1993, 1994a, 1995) and British Columbia (Kangasniemi 1983). Although all three taxa may have some potential to control milfoil, the work of Creed and Sheldon (1994a, 1995 and Sheldon and Creed 1995) suggests that *E. lecontei* is the most promising control agent. It also occurs more broadly and in greater abundance than the other taxa at the sites we have examined in Minnesota and Wisconsin (Newman and Maher 1995). Sheldon and O'Bryan (1996) and Newman et al. (1996a, 1997) describe the life history and development times of the weevil.

Declines of milfoil in several Vermont lakes have been correlated with the occurrence of *E. lecontei* (Sheldon and Creed 1995). Reductions in individual milfoil plant growth and mass due to *E. lecontei* adults (Creed and Sheldon 1993) and larvae (Creed and Sheldon 1994b) and reductions in plant buoyancy due to weevil feeding (Creed et al. 1992) have been demonstrated in small scale laboratory experiments. In pond enclosure experiments, significant reductions in milfoil growth and biomass due to weevil adults and larvae have been shown (Creed and Sheldon 1995, Sheldon and Creed 1995). Creed and Sheldon suggested that weevils, via larval stem boring, may reduce plant vigor by reducing plant buoyancy and light capture. They also hypothesized that larval stem damage, by inhibiting translocation of gases and nutrients, could stress the plants; specifically, they proposed that reductions in carbohydrate translocation to roots in the summer would lower over winter carbohydrate reserves resulting in overwinter declines or reduced growth in the spring. Experimental work showed substantial reductions of milfoil shoot and root biomass and carbohydrate stocks associated with weevil abundance (Newman et al. 1996a) and suggested that the mechanisms hypothesized by Creed and Sheldon (1995) may be operating. However, our results also suggested that densities of 200-300 weevils per m^2 may be needed to effect control (Newman et al. 1996a), levels that have not yet been documented in Minnesota lakes. Fish predation may be one factor limiting populations (Sutter and Newman 1997).

This project was aimed at determining the occurrence of field declines of milfoil, the dynamics of populations of the weevil *E. lecontei*, and identifying factors that may relate to effects of the weevil on Eurasian watermilfoil. The ultimate goal is to develop a mechanistic understanding why declines may or may not occur, based on control agent densities and limiting factors, site characteristics, and plant health or carbohydrate status.

This report will summarize our methods and results during 1995-1996 and provide a synthetic interpretation of the use of biological control for Eurasian watermilfoil with recommendations for future action. In addition we will report a few general results from samples collected in summer 1997, but detailed analyses of these results will be reported in our progress reports for the next biennium.

Deliverable 5. Final report.

Due Date: 15 November 1997 $13,500

Deliverable: Field analyses, including results of sampling done during June 1997 ($8,000), will be completed and included in a report summarizing the results of all objectives. An introductory overview of the purpose, scope and literature related to the project will be presented. Individual chapters containing the results of each objective will follow, and a synthetic analysis and interpretation of the combined results will be presented. Recommendations for the likelihood of continued success with invertebrate biocontrol agents will be presented along with a suggested course of future action. This will be the final report.

Acknowledgements

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Methods

Semi-permanent Transect Sites:

During the summer of 1993, we initiated selection of semi-permanent sampling sites, which can be repeatedly sampled at fixed locations (Newman and Ragsdale 1995). A site was chosen in Lake Auburn (Carver Co.; T116N; R24W; S10) in July and another site was chosen in Otter Lake (Anoka and Ramsey Co.; T30-31N; R22W; S3-4, S35-36) in October 1993. In spring 1994, we selected and sampled two additional semi-permanent sites: Cedar Lake (Hennepin Co.; T29N; R24W; S29) and Smith's Bay of Lake Minnetonka (Hennepin Co.; T117N; R23W; S10,11). At each site, 5 transects, 30 m apart, were run from near shore (1 m depth) toward the plant limit. At Lake Auburn and Cedar Lake, the transects were extended to 50 m from the shoreward starting point, in approximately 2.5 m depth at Auburn and 5 m depth in Cedar. Semipermanent stations were marked along the transect at 10 m intervals with florescent floats that were attached to bricks and suspended 0.5-1m beneath the surface. At Otter Lake, the transects were extended 100 m from shore, in approximately 2 m depth. At Smith's Bay, transects were started 100 m from shore and run to 4.5 m depth, approximately 0.8 km from shore, with 5 sampling stations along each transect approximately geometrically spaced. Distances from shore determined from GPS data were: 100m, 200m, 370m, 585m and 805m. These stations were marked with floating milfoil bouys.

In summer 1996, while looking for an introduction or augmentation site, we noticed a dense population of weevils a Cenaiko Lake (Anoka Co.; T31N; R24W; S26). We therefore sampled this lake in July and September as a new site to be regularly sampled. We ran 3 or 4 transects, west to east across the north end of the lake, with sampling stations every 30 m. This resulted in 25-32 samples on each date (21-30 with plants; deep stations were deleted from the analysis). At Lake Auburn transects were sampled at 10 m intervals (stations), resulting in 6 samples per transect, or 30 samples. At Otter Lake samples were taken at each 20m sampling station, resulting in 5-6 samples per transect or 27 samples. At Cedar (30) and Smiths Bay (25), all stations were sampled, however, several stations in Cedar Lake were deeper than the plant limit (>7m) and these are excluded if no plants occurred there during the season. In 1995-1996 sampling occurred in June, late July or early August and mid- to late-September. Samples were alternately taken 1m from each side of each station and then 2 m from each side of each station on successive sampling dates to minimize sampling disturbance. At each sampling station, plant biomass and invertebrate samples were taken from 0.1 m^2 quadrats (all plant material was clipped at sediment interface and immediately placed in a sealable bag underwater). Sediment cores, water samples (for chlorophyl a) and samples of milfoil (for carbohydrate analysis) were also collected at shallow, medium and deep stations along transects 1, 3 and 5 at each site.

A set of water column parameters were measured in the open water (>5.5m depth and >100 m from the bed) at each site on each sampling date. Secchi depth and surface conductivity were measured and a water sample (combined surface and Secchi depth sample) was collected for pH, alkalinity and chlorophyll a determination. A light (Photosynthetically active radiation = PAR, Li-Cor LI-189 with LI-192SA quantum sensor), temperature and oxygen (YSI 50B) profile was taken at 0.5 m depth increments from surface to bottom.

Alkalinity was determined by titration in the field. For chlorophyll, 500 ml of water were filtered through a 1.2 mm glass fiber filter, the filter was placed on dry ice and returned to the laboratory. Within 30 days, chlorophyll was extracted and measured spectrophotometrically (APHA 1989). Sediment cores were stored on ice and returned to the laboratory. Within 24 hr the top 15 cm of sediment was homogenized. A 5 ml sediment subsample was dried at 105 ˚C for 24-48 hrs and then weighed to obtain bulk density (g dry mass ml^{-1}). The dried sediment was then ashed at 550 ˚C for 4 hrs to obtain percent organic matter ([AFDM dry $mass^{-1}$] X 100). Pore water was extracted from the remaining sediment by centrifugation, acidified to < pH 2 and stored in the refrigerator. Within seven days, the NH_4 concentration was determined by selective electrode (APHA, 1989).

At least nine quadrat biomass samples from each site (selected on a stratified basis to coincide with the sediment cores) were generally processed for invertebrates, plant biomass and stem damage; the remaining samples were assessed for invertebrates and plant biomass, but stem damage was not measured. Biomass samples were rinsed of invertebrates and invertebrates were picked (endophytic and external on milfoil and from the wash water) from all samples and weevils were enumerated. Plants were separated, identified to species, spun for 15 sec in a salad spinner and wet mass was recorded. These samples were then frozen for later dry mass determination. For samples assessed for stem damage (leaf damage was not recorded), *M. spicatum* plants were measured and the total length of stem was recorded for each of the plants. Two randomly selected representatives of *M. spicatum* plants from each of three length classes were selected. These classes were defined as < 0.5m ("short"), 0.5m to 1.0m ("medium") and >1.0m ("long"). If there were no plants in a class, the total of six plants was taken from the other classes. These plants were measured for the length of stem damaged and the proportion of apical meristems (on main and lateral stems) which was missing. Subsample damage was extrapolated to the 0.1m^2 sample by multiplying the proportion of damage in the subsample stems in each length category by the total length of all stems in that category, summing over all three length categories and dividing by the total stem length in the sample (modified from Newman et al. 1993).

Survey Sites:

Four sites previously sampled in 1992 and 1993 were resampled for milfoil, invertebrates and site characteristics in late August or early September 1995 and 1996 to give a broader coverage of sites and to detect possible declines. The four sites chosen were Lake of the Isles (Hennepin Co.; T29N; R24W; S32,33), Shady Island (Hennepin Co.; T117N; R23W; S26) and Grays Bay (Hennepin Co.; T117N; R22W; S8) in Lake Minnetonka and Fish Lake (Dane Co., Wisconsin; T9N; R7E; S3). At each of these sites 3 transects were run perpendicular to shore and 3 stations, based on depth (e.g., 2, 3 and 4 m), were sampled along each transect. At each station 0.1m^2 quadrat samples were taken for plants and invertebrates. Sediment cores and milfoil roots and shoots (for carbohydrate analyses) were sampled at the intermediate depth station along each transect. Open-water water quality samples were taken and processed in the same manner as the permanent transect sites. Samples were processed as above for plant mass by species, weevil abundance, sediment characteristics and plant carbohydrates.

Weevil Introduction/Manipulation:

Our aim was to introduce known numbers of weevils to randomly selected plots at two sites. An equal number of randomly selected plots, without weevil introduction, would serve as controls. Because weevils likely exist in most lakes these experiments might be best termed augmentations. In 1995 the site chosen was on the northeast end of Otter Lake (Anoka Co.; T30N;R22W;S36). Ten plots were selected in dense milfoil; each plot was at least 70m apart. At each plot a center buoy was established and a 20 m transect, 10m on each side of the center buoy was laid out. Sampling stations at 2, 4 and 10m from the center were established on each side of the center buoy. At each plot one of the 10m transects was randomly chosen for pre-introduction sampling and milfoil biomass and weevils were sampled at each station with a 0.1m^2 submersed quadrat (all plants were collected by SCUBA from the quadrat and placed in sealable plastic bags). At the 4m station a sediment core and a sample of milfoil stems and roots (for carbohydrate analysis) were also collected. One week later, 5 plots were selected in a stratified random manner as controls and 5 plots were selected for weevil stocking. At each of the plots to be stocked, 100 weevil adults and 45 weevil larvae were stocked within 2m of the center buoy for a stocking density of ≈8 adults/m^2. Weevils to be stocked were collected from stock tanks on campus and Lake Auburn.

One week after introduction a visual survey was conducted at each plot along a 10m transect perpendicular to the biomass sampling transects. The plants along the 10m transect were examined for damage, weevils and weevil eggs by slowly snorkeling toward the center buoy during a period of 15min. Three weeks later (four weeks after stocking) the unsampled transect at each plot was sampled at 2, 4 and 10m for milfoil and weevils with the submersed quadrat. Cores and milfoil plants (for carbohydrate analysis) were again taken at the 4m station. This resulted in 3 quadrat samples and 1 sediment core at each of the 5 treatment plots and at the 5 control plots before stocking and four weeks after stocking for 5 replicates of control and treatment responses for each variable. Plant samples were refrigerated, rinsed of invertebrates, and invertebrates were picked from the plant samples. Plants were identified, spun in a salad spinner to remove excess water, and weighed. Weevils and lepidopterans were identified and enumerated. Cores were processed as above for pore water ammonia, bulk density and percent organic matter, and milfoil roots and shoots were analyzed for carbohydrates.

We intended to continue assessment in 1996 at the Otter site used in 1995 and to try augmentation at one other site. Unfortunately for further analysis, the Otter Eurasian watermilfoil population collapsed over the winter (see below) so this site was unusable for further assessment of augmentation. A second potential site, Stonewood Pond (Hennepin Co.) was found to have weevils and was thus unsuitable as an open introduction site. A third site, Cenaiko Lake, was found to have the highest weevil densities we have seen and was included with our permanent transect sites. Therefore, we decided to try augmentations in plots in Lake-of-the-Isles and Cedar Lake; we have good background data on both of these sites and weevils were in low density at Cedar Lake and had not been found in Lake-of-the-Isles until summer 1996 (at very low density).

The site chosen in Lake-of-the-Isles was between the big island and the north central peninsula (east of the north arm). Six plots were selected in dense milfoil; each plot was at least 70m apart. On Cedar Lake, 8 plots were located in the north east bay (southeast of our permanent transect site). At each plot a center buoy was established and three 10m transects extending from the center buoy were laid out. Sampling stations at 2, 4 and 10m from the center were established along each transect. At each plot one of the 10m transects was randomly chosen for pre-introduction sampling. Milfoil biomass and weevils were sampled in mid-July at each station with a 0.1m^2 submersed quadrat (all plants were collected by SCUBA from the quadrat and placed in sealable plastic bags). At the 4m station a sediment core and a sample of milfoil stems and roots (for carbohydrate analysis) were also collected. After sampling, 7 plots were selected in a stratified random manner as controls (3 in Isles and 4 in Cedar) and 7 plots were selected for weevil stocking. At each of the plots to be stocked, 60 weevil adults were stocked within 2m of the center buoy for an initial stocking density of ca. 5 adults/m^2. Two weeks later, an additional 40 adult weevils were added to each plot for a total of 100 adults or about 8/m^2. Weevils to be stocked were collected from Cenaiko Lake.

Two weeks after introduction a visual survey was conducted at each plot along one of the unsampled 10m transects. The plants along the transect were examined for damage, weevils and weevil eggs by slowly snorkeling toward the center buoy during a period of 15min. Visual surveys were also conducted five weeks and 7 weeks after stocking on a randomly selected transect. Two months after stocking an unsampled transect at each plot was sampled at 2, 4 and 10m for milfoil and weevils with the submersed quadrat. Cores and milfoil plants (for carbohydrate analysis) were again taken at the 4m station. Only samples at the 2 and 4m stations were processed. This resulted in 2 quadrat samples and 1 sediment core at each of the 7 treatment plots and the 7 control plots, before and 2 months after stocking (7 replicates of control and treatment responses for each variable). Plant samples were refrigerated, rinsed of invertebrates, and invertebrates were picked from the plant samples. Plants were identified, spun in a salad spinner to remove excess water, and weighed. Weevils and lepidopterans were identified and enumerated. Cores were processed as above for pore water ammonia, bulk density and percent organic matter, and milfoil roots and shoots were analyzed for carbohydrates.

Overwinter assessments:

The aquatic portion of the life cycle of the weevil has been described by Creed and Sheldon (1993) and Sheldon and O'Bryan (1996a). From early spring through late fall the weevil is found in the water on watermilfoil. To pass the winter, adults leave the aquatic environment in late fall and enter reproductive diapause (Sheldon and O'Bryan 1996a). Because there have been declines in Eurasian watermilfoil associated with high population densities and damage caused by weevil feeding and pupation (Sheldon and Creed 1995) there is an interest in understanding factors that may limit the weevil population both within the aquatic and terrestrial habitats. In the aquatic habitat there is some evidence that sunfish, *Lepomis* spp. are an important mortality factor for *E. lecontei* (Sutter and Newman 1997). Nothing was known about the terrestrial habitat and potential mortality factors that may impact weevil population dynamics. This study was initiated to characterize the biology of *E. lecontei* while it resides in the terrestrial habitat.

Collection of Adult Weevils for Augmentative Release: Adult weevils were collected Fall, 1995 from two habitats, from milfoil shoot tips in approximately 1m of water at Lake Auburn between mid-October to late-September and from soil samples taken at Smith's Bay on 6 November. Collection of adult weevils from milfoil in the late fall is far easier than sifting through soil samples. We estimate that in the same amount of time used to process soil samples, 20 times the number of insects can be collected from milfoil in the late fall. In previous work we demonstrated that adult weevils collected from soil samples could be held at 4 °C for several months with excellent survival. The objective of this study was to determine if weevils collected from the water in late fall at Lake Auburn could be stored at 4 ˚C as readily as those collected from soil samples. The purpose of storing insects over the winter is for subsequent augmentative releases where milfoil is present but weevils are absent or rare.

We used weevils from two different locations for this study. Weevils collected in the water were from Lake Auburn (Carver County) and weevils taken from soil samples were from Smith's Bay - Lake Minnetonka (Hennepin County). The source of weevils was not likely a major source of experimental error as data taken on overwinter survival from these two sites have been nearly identical both in field sampling and in laboratory studies. We used weevils collected in soil samples from Smith's Bay because of the higher density of weevils known to be present at this location.

Weevils from both locations were brought back to the laboratory, sorted and placed in 50ml capped tubes filled with soil having approximately 10% moisture within a few days after collection (1-2 days for weevils collected from water and 1-8 days for weevils in soil samples. Collected weevils were placed on the soil surface and allowed the opportunity to find a suitable site by leaving tubes in the laboratory overnight before placing them in 4 ˚C. Survival was measured on 3 April 1996.

Adult Dispersal. A persistent question has been how weevils disperse in either Fall or Spring. We have attempted to monitor movement of weevils via pit fall traps, clear "window-pane" traps and indirectly by taking soil samples at intervals away from the shoreline. None of these sampling methods have given us a good indication of weevil dispersal. A few weevils have been collected by all these sampling methods but no consistent pattern has been discovered. Weevils in the Fall and Spring are capable of flight based on dissection of collected adults. Adults have fully developed flight muscles and have taken flight in the laboratory. In contrast weevils collected mid-summer from milfoil have much reduced flight muscles and cannot be induced to fly, so that mid-summer dispersal appears not to occur.

An additional sampling method was used Spring 1996. We modified yellow Japanese beetle traps after first testing their efficacy in the laboratory. No weevil escaped from the traps in the laboratory. Other members of the Phytobiini weevils are daytime fliers (rather than night fliers as are many weevils) and would likely be attracted to yellow (O'Brien, personal communication). We placed 3 modified traps along the shore (approximately 50m apart) with a corresponding trap in the water (approximately 10m out from shoreline trap) at Lake Auburn and Smith's Bay. Traps were checked on a weekly basis.

Shoreline surveys: Periodic samples have been taken on several lakes in Minnesota and Wisconsin. Spring and fall shoreline soil litter (overwinter) samples were collected at Smith’s Bay and Lake Auburn from 1993 - 1997. In fall 1996, Cenaiko Lake was added to our shoreline collection sites. Shoreline soil samples consist of the top 2.5-7.6 cm of soil and leaf litter in a 0.2 m^2 area area. Samples were returned to the laboratory, dried if needed, sifted through a series of gradually decreasing sieves and examined for the presence of weevil adults. Litter sampling was used to estimate weevil population density, timing when insects move into the terrestrial habitat and when they emerge in the spring to recolonize the aquatic habitat.

Weevil development with temperature:

To determine the relationship between weevil development time and temperature weevils were reared from egg to adult in growth chambers at 5 temperatures: 15, 19, 23, 27 and 31 ˚C. Eurasian watermilfoil plants were started in outdoor tanks and allowed to root and grow to a length of ca 35 cm. A female weevil (one of 12 mated females randomly assigned to plants) was allowed to oviposit on a plant and the plant was then transferred to an individual acrylic tube (7.5 cm diameter, 45 cm tall) and rooted in lake sediment. The tube was filled with 20 ˚C well water. The tube was then randomly assigned to a temperature (environmental chamber). Sixteen such tubes were assigned to each chamber in a stratified procedure, to ensure that each female had one egg per temperature and that eggs were started in each chamber at about the same time. Plants were examined within the tubes daily and developmental stage was noted. Hatch was determined when the egg disappeared, followed by subsequent typical stem damage; larvae burrow through the stem as they continue development. Pupation was noted upon formation of a characteristic puparium and adults were captured and weighed as soon as they emerged from the puparium. Development times were determined for each stage and development rate (1/d) was calculated along with the threshold temperature for development. Additional temperatures (21, 25 and 29 ˚C) were assessed in summer 1997; these results fit well with the 1996 data and will be reported in our first progress report for the next biennium.

Genetic variation in weevil populations in relation to host-plant:

The genetic contribution to variation in hostplant-preference behavior is unknown (Bernays and Chapman 1994), however, *E. lecontei* has demonstrated intraspecific variation in hostplant preference when reared on different plant species (Solarz and Newman 1996). It is important to estimate the genetic and environmental components of variation in traits such as hostplant preference in order to determine the potential for an herbivore to adapt to novel plant species. In the summer of 1996, we conducted an experiment to determine the source of variation in hostplant preference, development time, and size at maturity among progeny of weevils collected from both Eurasian and northern source populations. The sources of variation to be determined with our design, include additive genetic variation (V_A), maternal and dominance effects (V_M and V_D), phenotypic plasticity, the interaction of V_A with different environments (V_A x E), and the interaction of V_M with different environments (V_M x E).

Weevils were collected from two source populations. One population was from *M. spicatum* (Fish Lake, Sauk City, Wisconsin and one from *M. sibiricum* in a lake lacking *M. spicatum* (Christmas Lake, Hennepin County, Minnesota). Eight males from each of the two populations were mated to 24 females in a nested hierarchical design (e.g. Mazer and Wolfe 1992) which produced 576 progeny. The resulting progeny were each randomly assigned to one of four rearing treatments: 1. & 2. Eurasian or northern throughout development, 3. & 4. switched mid-development either from Eurasian to northern or vice versa. Upon emergence of the progeny, development time was recorded and oviposition preference and body length and width was taken for dams, sires, and progeny. Development time, oviposition preference, and morphological data were analyzed as a nested factorial mixed model (Montgomery 1991; Milliken and Johnson 1992; R. Shaw, University of MN, pers. comm.) using a Restricted Maximum Likelihood Analysis (REML; Shaw and Shaw 1992). From the REML results, we partitioned the variation among each of the main and interaction effects, thus determining the relative importance of additive effects, dominance and maternal effects, environmental effects, and genotype x environment interactions.

Results and Discussion

Semi-permanent transect sites:

Milfoil biomass in in June 1995 was higher than the previous summer at all lakes except Smith's Bay (Table 1; Fig. 1). Milfoil increased throughout the summer at Lake Auburn to the highest density we have recorded (>5000 g wet/m^2) and milfoil also remained dense in Otter Lake. Milfoil in Cedar Lake showed a summer long decline, likely due to poor water clarity. Milfoil in Smith's Bay continued a summer-long decline from levels in 1994. Eurasian watermilfoil in 1996 was generally lower than on similar dates in 1995, with the exception of Lake Auburn (Table 1; Fig. 1). Milfoil crashed over winter at Otter Lake, dropping 100 fold from 2600 g/m^2 (wet mass) in September 1995 to 21g/m^2 in June 1996. This decline was apparently due to a severe anoxic winterkill (a fish kill was noted in spring), however, the native plant community was not severely affected (Fig. 2), retaining a spring biomass of 430 g/m^2

(Table 2). Milfoil also decreased greatly at Smith's Bay, from a previous low of 1280 g/m^2 in September 1995 to 665 g/m^2 in June 1996. The non-milfoil plant community increased at Smith's Bay to 1160 g/m^2 or 67% of total plant biomass (Tables 2 and 3), however, *Potamogeton crispus* was dominant with a density of 694 g/m^2 (Appendix 1). June 1996 milfoil biomass at Cedar Lake was also much lower (570 g/m^2) than in June 1995 (2307 g/m^2), however this was an increase from September 1995 (479 g/m^2) when milfoil had declined over the summer due to poor water clarity. Water clarity was excellent in 1996 (Secchi Disk readings ranged from 5.5m in June to 1.9m in August) and about 1m deeper than in 1995 (Table 4), so the low density of milfoil in June was not due to poor water clarity. Milfoil in Lake Auburn continued a several year trend of increases (between 2900 and 3600 g/m^2), but did not reach the high density of over 5000 g/m^2 reached in September 1995.

Table 1. Biomass ± 1SE (g wet/m^2) of Eurasian watermilfoil at the four sampling sites in 1994-1997. n = number of samples.

Sampling Date	Auburn	n	Cedar	n	Otter	n	Smith's Bay	n
5/19-6/3/94	1474 ± 326	10	610 ± 289	18	2208 ± 332	21	1470 ± 320	14
7/1-7/11/94	1570 ± 297	16	1642 ± 523	18	1589 ± 231	27	3478 ± 399	16
8/12-8/19/94	1581 ± 224	15	601 ± 207	15	2626 ± 472	14	1886 ± 328	16
9/14-9/21/94	2205 ± 350	19	824 ± 188	24	2510 ± 557	9	1767 ± 386	14
6/07-6/27/95	1999 ± 324	30	2307 ± 631	23	3444 ± 336	27	1618 ± 289	25
7/31-8/15/95	2277 ± 417	19	1821 ± 797	10	2526 ± 385	15	1481 ± 245	25
9/18-9/29/95	5044 ± 752	17	479 ± 173	17	2629 ± 323	18	1281 ± 178	25
6/12-6/24/96	2959 ± 402	30	568 ± 200	30	21 ± 8	27	665 ± 144	25
7/30-8/9/96	2898 ± 625	27	665 ± 219	30	1 ± 1	27	1415 ± 256	25
9/12-9/19/96	3622 ± 469	30	574 ± 174	30	0 ± 0	27	1656 ± 393	25
6/27-7/17/97	2134 ± 321	30	1906 ± 341	28	24 ± 22	26	1880 ± 327	25
9/8-9/18/97	2786 ± 400	30	2646 ± 502	29	4 ± 4	27	1055 ± 170	25

Table 2. Mean number of species per sample (Spp/S) ± 1SE and non-milfoil biomass (B; g wet /m^2) at the 4 sampling sites in 1994-1997. Number of samples is given in Table 1.

Sampling Date	Auburn		Cedar		Otter		Smith's Bay	
	Spp/S	B	Spp/S	B	Spp/S	B	Spp/S	B
5/19-6/3/94	3.80±0.47	670	1.33±0.28	75	4.76±0.19	600	3.29±0.22	1231
7/1-7/11/94	3.63±0.29	444	1.83±0.28	370	4.37±0.29	520	3.75±0.35	1604
8/12-8/19/94	3.00±0.28	647	1.53±0.26	282	5.57±0.39	1126	3.13±0.42	765
9/14-9/21/94	3.11±0.37	268	1.46±0.19	54	4.89±0.61	431	3.50±0.39	975
6/07-6/27/95	2.23±0.22	822	1.43±0.20	214	4.70±0.21	1065	3.64±0.30	877
7/31-8/15/95	3.37±0.26	1789	1.70±0.15	516	4.27±0.30	642	2.68±0.24	703
9/18-9/29/95	2.18±0.18	1058	1.41±0.17	337	2.44±0.34	135	2.80±0.20	856
6/12-6/24/96	2.93±0.24	1450	2.10±0.22	248	5.19±0.25	434	4.32±0.36	1159
7/30-8/9/96	2.78±0.31	2070	1.43±0.18	270	4.19±0.20	1171	3.88±0.41	1017
9/12-9/19/96	2.50±0.20	1176	1.57±0.16	307	3.93±0.28	1798	3.88±0.32	1531
6/27-7/17/97	2.97±0.14	1435	1.82±0.14	460	4.31±0.29	1516	4.16±0.39	1162
9/8-9/18/97	2.63±0.17	1500	1.59±0.09	235	4.81±0.26	3180	3.64±0.27	1863

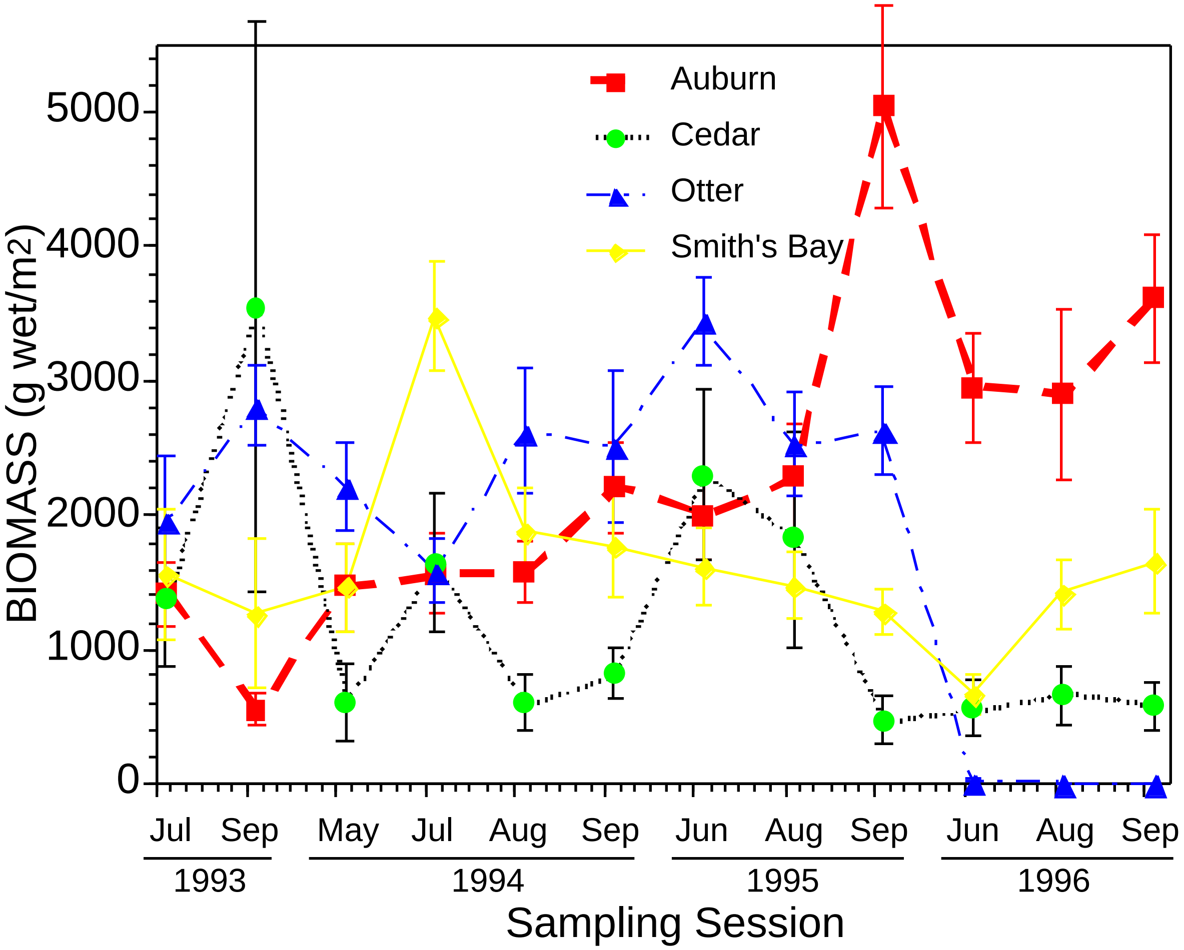

Fig. 1. Milfoil biomass (g wet/m^2 ± 1 SE) from the four field sites from July 1993 through September 1996.

Table 3. Percentages of total plant wet biomass that was Eurasian watermilfoil (±1SE) and number of species (N) collected at each site. These percentages are the average percentage found in the samples and are thus not equal to total mean milfoil biomass/plant biomass.

Sampling Date	Auburn	N	Cedar	N	Otter	N	Smith's Bay	N
5/19-6/3/94	65% ±10%	9	67% ±11%	4	80% ± 6%	9	64% ±10%	8
7/1-7/11/94	79% ± 6%	9	67% ± 9%	4	75% ± 5%	9	72% ± 6%	11
8/12-8/19/94	74% ± 6%	9	61% ±13%	3	75% ± 6%	11	81% ± 5%	11
9/14-9/21/94	91% ± 6%	9	87% ± 5%	4	83% ± 6%	11	71% ± 8%	9
6/07-6/27/95	72% ± 7%	7	82% ± 7%	3	79% ± 4%	9	61% ± 5%	10
7/31-8/15/95	58% ± 7%	7	58% ± 6%	2	80% ± 7%	9	63% ± 6%	11
9/18-9/29/95	81% ± 7%	5	38% ± 5%	2	95% ± 1%	6	63% ± 7%	10
6/12-6/24/96	70% ± 8%	7	57% ± 7%	5	7% ± 5%	9	33% ± 6%	10
7/30-8/9/96	54% ± 8%	7	59% ± 9%	5	0.1% ± 0.1%	10	56% ± 7%	11
9/12-9/19/96	69% ± 6%	8	73% ± 6%	4	0% ± 0%	9	49% ± 7%	10
6/27-7/17/97	53%± 13%	10	82% 9%	3	1.2% ± 2.3%	12	54% ± 14%	12
9/8-9/18/97	60% ± 13%	8	88% 9%	2	0.2% ± 0.3%	13	40% ± 14%	11

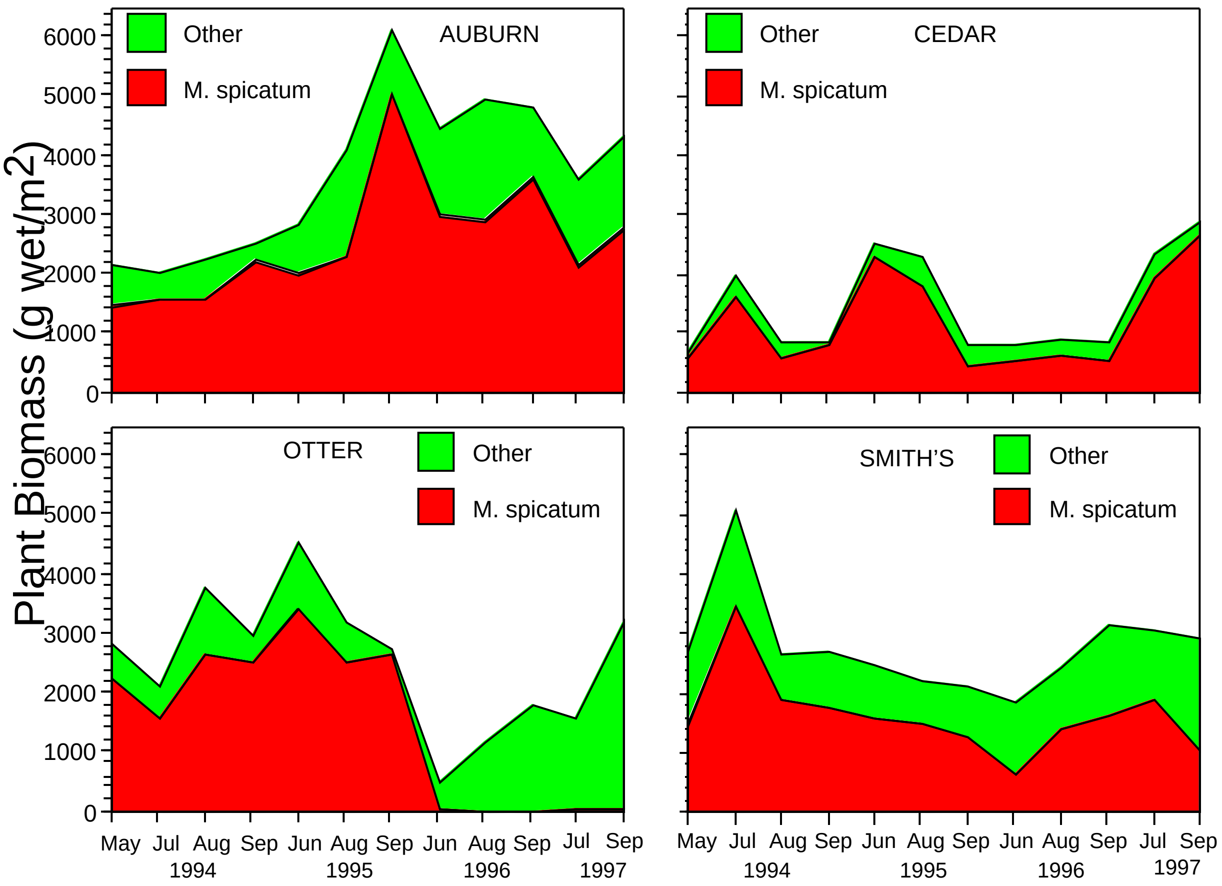

Fig. 2. Total plant biomass (milfoil and non-milfoil biomass; g wet/m^2) at the four permanent transect sites from May 1994 - September 1997.

During the summer of 1996, milfoil continued to decline in Otter Lake (Table 1); although a few scattered healthy plants were seen, no Eurasian watermilfoil was found in the 27 samples taken in September 1996. The native plant community increased throughout the summer and approached 2000 g/m^2 in September 1996; native plants even more abundant in 1997 (Table 2; Appendix 1). Milfoil increased in density in August and September at Smith's Bay, but remained below the densities observed prior to 1995 and the native plant community composed around 50% or more of biomass all summer (Table 3; Fig. 2). The slow decline at Smith's Bay continued through 1997 while native plants increased in biomass, to over 50% of total plant biomass. In contrast to previous years, milfoil at Cedar Lake in 1996 failed to increase above 1000 g/m^2, despite the good water clarity, however, it also did not decline in the fall, again, likely due to the good summer-long water clarity which was associated with low rainfall (depth at Cedar Lake dropped about 0.5m over the summer). Native plants appeared to respond to the better water clarity and lack of milfoil increase; more species were present in 1996 than the previous two years (Tables 2 and 3), but densities did not exceed 310 g/m^2. Milfoil at Lake Auburn increased to 3622 g/m^2 in September 1996, which is lower than September 1995, but is the second highest density recorded there. However, native species fared better at Auburn than in previous years, maintaining densities over 1000 g/m^2 throughout the summer and the highest levels since 1993 (Table 2).

Changes in milfoil biomass at our sites (Figs. 1 and 2) were not primarily due to regional changes; there was little concordance among the sites. Similarly, changes in milfoil biomass were not always in the same direction as other members of the plant community. For example, Eurasian watermilfoil became a lower proportion of the total plant biomass in Smith's Bay, Lake Auburn and Otter Lake in 1996 relative to 1995, but increased in proportion in Cedar Lake (Table 3). Species richness stayed the same (Smith's Bay) or increased at all sites in 1996 relative to 1995 (Tables 2 and 3). Smith's Bay retained 10-11 species throughout the summer while Lake Auburn retained 7 species. Five species were noted in Cedar Lake in June and August, the most for several years. In general, total species found paralleled mean number of species per sample (compare Tables 2 and 3). The declines of milfoil at Smith's Bay and Otter Lake been accompanied by an increase in other plants (Fig. 2; Table 2; Appendix 1), and even in Lake Auburn the native plant density in 1996 was higher than previous years despite the higher density of Eurasian watermilfoil. However, at Lake Auburn, as with Cedar Lake, the mean number of species per sample has remained much lower than at Smith's Bay and Otter Lake (Table 2). It should also be noted that northern watermilfoil reappeared at all of our sites in 1996 (seen but not sampled at Otter Lake), the first time it has occurred at these sites since early 1994. Northern watermilfoil was generally limited to shallow water, however.

Data for 1997 will be discussed in our first progress report for the 1997-1999 biennium, however, it should be noted that in 1997 the Eurasian watermilfoil remained at low densities in Otter Lake (<2% of biomass) and native plants increased to over 3000 g/m^2 (Table 2; Appendix 1). Eurasian watermilfoil continued a slow decline in Smith's Bay, to the lowest fall level yet recorded, while native plants increased to their highest levels, about 1800 g/m^2 (Table 2). Eurasian watermilfoil contribution to fall percentage of plant mass has declined each year since 1993. In contrast, Eurasian watermilfoil densities in Lake Auburn were only slightly lower than previous years and milfoil biomass increased substantially at Cedar Lake, persisting throughout the summer, likely due to increased water clarity associated with alum treatments in fall 1996.

Sediment characteristics at each of the lakes were similar in 1995 and 1996, with the exception of Smith's Bay, where sediment ammonium concentrations were about 60% of those in 1995 (Table 4). It is possible that the denser native plant communities kept sediment ammonium levels lower in 1996; ammonium levels did not increase in Otter Lake with the major decline in milfoil, again, likely due to the abundance of native plants. Ammonium levels did increase each September in Cedar Lake, possibly due to the low abundance of milfoil and other plants. Sediment bulk densities tended to decrease from spring to fall, partly associated with increasing percent organics, but this was not consistent among lakes.

Weevil densities were generally higher at our sites in 1996 than in 1995 and we also found more caterpillars (primarily *Acentria ephemerella*, but also *Parapoynx* sp.) than we have in the past (Table 5). Weevils were not detectable in our samples in Otter Lake after the milfoil decline in 1996, due to the very low density of milfoil present. We did notice weevils on milfoil plant in Otter Lake and in August 1996, found 3 adult weevils on 8 plants collected for carbohydrate analysis (0.4 weevils per plant). Weevil densities at Smith's Bay and Lake Auburn were not as high as those reached in summer 1994, however September 1996 densities exceeded September 1994 and 1995 densities, suggesting the potential for good overwinter densities. Weevils reached detectible levels in Cedar Lake in 1996 for the first time since July 1994, but densities remained low through the summer.

As we previously reported for 1994 and 1995, (Newman et al. 1996b), weevils were found at all stations in Lake Auburn in 1995 and 1996, although densities were highest at the 20-50m from shore stations. However, weevils apparently did not make it to the deepest and farthest station at Smith's Bay (4.5m; 805 m from shore) in 1994-1996. Weevils were most abundant at the shallowest three stations (<3m depth, < 400m from shore), but were relatively common at the 585 m stations, which are about 3.5m deep. Elimination of the deepest sites at Smith's Bay would raise weevil densities 25% and density of weevils at the stations <400m from shore were about 1.5 times higher than the overall density in Smith's Bay. These densities are likely exerting control in the shallower water whereas other factors appear to be limiting the milfoil in water > 3m deep.

Table 4. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1995 and 1996 at the four permanent transect sites. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 3 and 5 (n=9). Secchi depth (SD), chlorophyll a (Chl-a; pooled surface and SD sample) and light and temperature profiles were taken in deep water > 100 m from the plant bed. Temperature is at 1m depth and 10% PAR depth is the depth at which light intensity was 10% of surface light (presented as the range which encompassed the 10% value).

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/ml)	SD (m)	Temp (˚C 1m)	10% PAR Depth (m)	Plant Limit (m)
Auburn								
6/15/95	0.60	3.96	11.34	9.5	2.3	20.7	2.5-3.0	3.0
2se	0.15	0.91	3.73					
8/1/95	0.49	4.00	10.69	13.9	1.4	26.0	1.5-2.0	3.0
2se	0.18	1.24	4.39					
9/26/95	0.45	4.40	12.67	8.0	2.0	14.8	2.5	3.0
2se	0.13	1.96	4.05					
6/13/96	0.41	3.08	16.0	2.9	4.2	25.1	3	3.0
2se	0.11	1.66	8.6					
7/31/96	0.42	5.81	13.6	12.8	2.4	23.3	1-1.5	3.0
2se	0.17	1.52	4.7					
9/12/96	0.38	2.68	13.7	8.8	2.35	21.2	2.5-3.0	3.0
2se	0.14	0.95	4.3					
Cedar								
6/28/95	0.62	3.90	13.73	10.2	4.5	24.0	4.5	4.0
2se	0.36	1.63	6.00					
8/3/95	0.45	7.27	16.41	16.3	1.2	26.7	1.0-1.5	3.1
2se	0.33	1.39	7.40					
9/28/95	0.43	6.06	21.56	27.5	0.75	14.8	1.0-1.5	3.1
2se	0.36	1.98	7.38					
6/18/96	0.57	3.78	13.3	1.1	5.5	24.6	3.5-4.0	6.5
2se	0.38	1.34	6.3					
8/1/96	0.42	3.86	19.0	4.5	1.9	23.8	2.5-3.0	3.1
2se	0.38	1.59	7.5					
9/16/96	0.41	5.12	18.5	5.3	2.8	20.1	2-2.5	3.1
2se	0.37	1.63	6.9					
Otter								
6/26/95	0.42	3.27	20.26	5.6	3.0	30.0	3.5-4.0	4.0
2se	0.18	1.43	7.23					
8/10/95	0.39	4.66	24.44	12.5	2.5	24.7	1.5-2.0	4.0
2se	0.26	1.77	9.49					
9/30/95	0.38	2.76	25.07	3.7	1.1	14.5	1.0-1.5	4.0
2se	0.26	1.34	11.34					
6/20/96	0.47	4.86	23.5	8.5	1.9	21.1	1.5-2.0	3.5
2se	0.34	1.67	10.2					
8/6/96	0.27	3.54	27.5	4.8	2	26	2-2.5	4.0
2se	0.16	0.88	8.6					
9/17/96	0.33	3.77	24.9	8.0	1.5	17.9	1.5-2.0	4.0
2se	0.24	1.76	9.5					
Smith's								
6/29/95	0.59	5.18	11.81	4.0	3.9	23.7	5.0	5.0
2se	0.25	3.40	4.62					
8/16/95	0.28	4.06	12.86	7.5	2.1	24.9	3.5-4.0	5.0
2se	0.14	0.97	3.71					
9/18/95	0.31	4.25	12.50	10.7	2.1	14.7	2.5	5.0
2se	0.15	0.77	3.98					
6/24/96	0.36	1.13	13.9	3.7	3.7	20.6	3.5-4.0	5.0
2se	0.22	0.32	4.7					
8/8/96	0.37	2.61	17.6	1.3	3.4	24.4	4.5-5.0	5.0
2se	0.21	1.01	5.3					
9/19/96	0.32	2.43	19.1	3.2	3.5	20.1	3.0-3.5	5.0
2se	0.18	0.90	14.3					

Table 5. Density (N/m^2 ± 2 SE and N per stem ± 2SE) of *Euhrychiopsis lecontei* larvae, pupae and adults and *Acentria ephemerella* at the four permanent transect sites, 1994-1996. In 1996, some *Parapoynx* were found and are lumped with *Acentria.** or *Parapoynx* dominated sample**. A stem is a basal milfoil stem emerging from the sediment; estimates per stem do not include samples without milfoil.

Lake	Date	Weevil n	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2
Auburn	May-94	9	27.8 ±27.4	1.1± 2.2	6.7± 8.8	35.6± 36.5	1.1± 2.2
	per stem	9	0.134±0.103	0.002±0.004	0.018±0.020	0.154±0.106	
	Jul-94	16	58.8± 21.1	12.5± 9.6	31.3± 14.0	102.5± 36.7	6.3± 7.7
	per stem	16	0.217±0.092	0.034±0.034	0.084±0.036	0.335±0.127	
	Aug-94	15	8.7± 7.5	2.0± 2.9	3.3± 3.7	14.0± 9.5	0.7± 1.3
		15	0.031±0.025	0.003±0.005	0.008±0.008	0.042±0.030	
	Sep-94	18	1.7± 3.3	2.2± 2.6	7.8± 7.8	11.7± 11.8	3.9± 3.3
		18	0.002±0.004	0.006±0.008	0.014±0.012	0.022±0.019	
	Jun-95	30	6.0± 4.0	0.7± 0.9	1.0± 1.1	7.7± 2.7	0.3± 0.7
		21	0.070±0.043	0.003±0.006	0.011±0.015	0.085±0.056	
	Jul-95	15	2.0± 2.1	0.7± 1.3	5.3± 5.5	8.0± 3.8	0.0± 0.0
		14	0.006±0.009	0.000±0.000	0.032±0.039	0.038±0.042	
	Sep-95	16	2.5± 2.2	3.1± 3.5	3.8± 4.0	9.4± 3.4	1.3± 1.7
		11	0.140±0.194	0.049±0.090	0.103±0.180	0.292±0.385	
	Jun-96	30	31.0± 17.8	2.0± 2.0	0.0± 0.0	33.0± 19.5	0.3± 0.7
		27	0.729±1.179	0.080±0.148	0.000±0.000	0.809±1.326	
	Jul-96	25	9.2± 15.2	3.6± 2.6	12.8± 6.3	25.6± 17.9	2.4± 1.5*
		23	0.029±0.043	0.020±0.021	0.048±0.027	0.096±0.061	
	Sep-96	30	6.7± 4.3	2.3± 1.6	3.0± 2.7	12.0± 6.5	6.3± 4.4
		29	0.048±0.053	0.007±0.005	0.011±0.010	0.065±0.055	
Cedar	May-94	11	5.5± 10.9	0.0± 0.0	0.9± 1.8	6.4± 10.9	0.0± 0.0
		0	–	–	–	–	
	Jul-94	14	4.3± 8.6	1.4± 2.9	1.4± 2.9	7.1± 14.3	0.0± 0.0
		0	–	–	–	–	
	Aug-94	11	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
	Sep-94	17	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
	Jun-95	18	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
	Aug-95	10	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
	Sep-95	17	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
	Jun-96	29	0.3± 0.7	0.0± 0.0	0.0± 0.0	0.3± 0.7	0.0± 0.0
		25	0.010±0.020	0.000±0.000	0.000±0.000	0.010±0.020	
	Aug-96	21	0.0± 0.0	0.5± 1.0	0.5± 1.0	1.0± 1.9	0.0± 0.0
		21	0.000±0.000	0.002±0.004	0.002±0.004	0.004±0.008	
	Sep-96	23	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
		24	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	

Table 5. Continued.

Otter	May-94	20	12.5± 10.2	0.0± 0.0	0.0± 0.0	12.5± 10.2	0.5± 1.0
		20	0.047±0.038	0.000±0.000	0.000±0.000	0.047±0.038	
	Jul-94	24	0.4± 0.9	0.0± 0.0	0.4± 0.9	0.8± 1.2	0.0± 0.0
		24	0.001±0.002	0.000±0.000	0.001±0.003	0.002±0.003	
	Aug-94	14	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	1.4± 2.9
		14	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	
	Sep-94	8	0.0± 0.0	1.3± 2.5	2.5± 3.3	3.8± 3.7	6.3± 5.3
		7	0.000±0.000	0.003±0.007	0.013±0.022	0.016±0.021	
	Jun-95	27	5.9± 5.1	2.6± 3.3	3.3± 3.4	11.9± 9.0	0.4± 0.7
		26	0.033±0.030	0.021±0.034	0.022±0.020	0.076±0.071	
	Aug-95	15	0.0± 0.0	0.0± 0.0	0.7± 1.3	0.7± 1.3	0.0± 0.0
		1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	
	Sep-95	18	0.6± 1.1	0.0± 0.0	1.1± 2.2	1.7± 2.4	0.0± 0.0
		1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	
	Jun-96	25	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
		5	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	
	Aug-96	26	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	3.1± 2.4
		2	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	
	Sep-96	27	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	104.8± 25.9**
		0	–	–	–	–	
Smith's Bay	Jun-94	13	3.8± 5.3	0.0± 0.0	0.8± 1.5	4.6± 6.6	0.0± 0.0
		12	0.020±0.030	0.000±0.000	0.005±0.010	0.025±0.040	
	Jul-94	11	12.3± 13.0	6.9± 8.0	1.5± 2.1	20.8± 20.9	0.8± 1.5
		13	0.064±0.083	0.038±0.052	0.006±0.009	0.108±0.137	
	Aug-94	16	18.0± 15.0	3.1± 4.0	1.9± 2.7	23.1± 20.2	0.6± 1.3
		15	0.104±0.079	0.019±0.022	0.010±0.015	0.133±0.109	
	Sep-94	14	0.0± 0.0	1.4± 2.9	2.1± 2.3	3.6± 4.5	0.0± 0.0
		14	0.000±0.000	0.003±0.006	0.013±0.020	0.016±0.022	
	Jun-95	25	0.4± 0.8	0.0± 0.0	0.8± 1.1	1.2± 1.3	0.0± 0.0
		14	0.001±0.003	0.000±0.000	0.027±0.048	0.028±0.047	
	Aug-95	25	4.0± 4.3	1.2± 1.8	0.4± 0.8	5.6± 5.3	0.0± 0.0
		9	0.080±0.096	0.000±0.000	0.007±0.015	0.087±0.107	
	Sep-95	25	0.8± 1.1	2.0± 3.3	0.8± 1.1	3.6± 5.0	0.0± 0.0
		15	0.010±0.014	0.025±0.039	0.013±0.019	0.048±0.061	
	Jun-96	25	4.8± 5.8	0.0± 0.0	0.0± 0.0	4.8± 5.8	5.2± 8.8
		20	0.037±0.043	0.000±0.000	0.000±0.000	0.037±0.043	
	Aug-96	25	12.4± 10.0	1.2± 1.8	2.0± 2.0	15.6± 10.5	1.6± 2.5*
		24	0.107±0.084	0.006±0.008	0.015±0.015	0.127±0.087	
	Sep-96	25	1.2± 1.8	2.0± 2.0	2.8± 3.4	6.0± 5.3	0.8± 1.1
		24	0.005±0.007	0.009±0.009	0.014±0.015	0.028±0.022	

Weevil density per basal stem was calculated (Table 5) for comparison with other studies. Weevils per stem generally parallels the areal densities, however, because stem densities vary, the two measures are not directly comparable. The highest densities per stem were found in Lake Auburn, with high densities of 0.3/stem in September 1995 and 0.8/stem in June 1996 (Table 5). Densities in Smith's Bay were lower, ranging from 0.03/stem in June 1995 and September 1996 to 0.13/stem in August 1996. Densities in Cedar and Otter Lakes were always <0.1/stem.

Cenaiko Lake

We unfortunately did not examine Cenaiko Lake before early July 1996, however at that time milfoil was matted on the surface over most of the northern littoral area of the lake. The milfoil was very damaged (necrotic stems) and supported a high weevil density. Adult weevil densities were the highest we have seen (Table 6) and weevils were collected from Cenaiko for use in our augmentation plots. Densities in July totaled 103/m^2, slightly higher than the highest density we recorded in Lake Auburn, in July 1994. Density per stem was also much higher than at the other sites: 1.6/stem in July 1996. We also found the highest density of *Acentria* we have encountered, over 30/m^2. It is likely that by July weevil densities were already decreasing due to the low density and poor condition of the milfoil; by September, weevil densities had declined to 8/m^2 (but still 0.7/stem; Table 6). Milfoil biomass at Cenaiko declined from 974g/m^2 in July to 239g/m^2 in September 1996 (Fig. 3). Given that the milfoil was already heavily damaged before we initially sampled in July, we suspect that the June biomass would have approached 2000g/m^2. While milfoil declined, native plant biomass increased from 938g/m^2 to 1374 g/m^2 (Appendix 2), or 67% of plant biomass in September 1996. The milfoil decline persisted through 1997 with densities of 8 and 30 g/m^2 in July and September, compared to native plant biomass of 1117 and 1017 g/m^2 in those months.

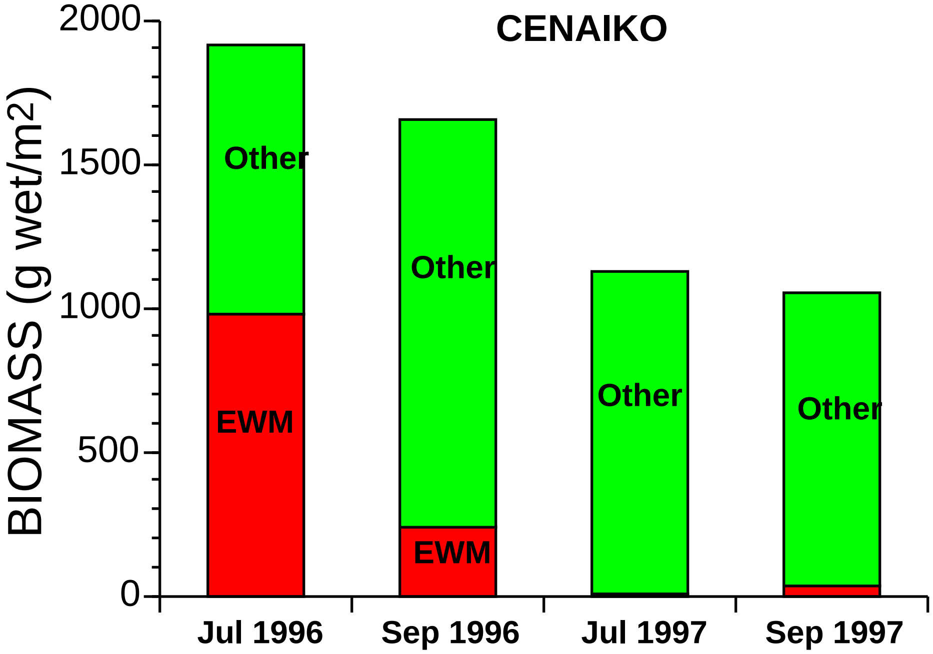

Fig. 3. Biomass of milfoil (EWM) and non-milfoil plants at Cenaiko Lake, 1996-1997. Milfoil declined significantly between July and September 1996 and July 1997 and a significant increase in native plants in 1996. N > 20 samples on each date. Mean number of species per sample (± 1SE) from Jul 1996-Sep 1997 respectively was: 2.31±0.19, 1.96±0.25, 1.90±0.23 and 1.89±0.23.

Table 6. Density (N/m^2 ± 2 SE and N per stem) of *Euhrychiopsis lecontei* (*E.l.*) larvae, pupae and adults, and *Acentria ephemerella* and *Parapoynx* sp. at Cenaiko Lake in 1996. Densities per stem were only calculated for samples with Eurasian watermilfoil. A stem is a basal milfoil stem emerging from the sediment.

Date	Weevil n	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx* N/m^2
7/22/96	29	48.6± 25.2	22.8± 10.8	31.7± 13.6	103.1± 41.9	18.3± 7.7	1.0± 1.5
per stem	26	0.923±1.292	0.337±0.458	0.381±0.280	1.640±1.972		
9/5/96	21	2.9± 2.4	1.0± 1.3	4.3± 4.3	8.1± 5.6	31.9± 20.2	0.0± 0.0
per stem	8	0.229±0.259	0.008±0.017	0.417±0.516	0.654±0.721		

Cenaiko Lake is somewhat different from our other sites; it is a human-made lake that is stocked with trout. Water clarity was high (Table 7), but the sediment had little organic matter and a high bulk density; much of the lower material was clay, possibly as a clay liner, and it was difficult to obtain cores that extended 10cm into the sediment. The existing sediment appeared to be contributed from bank erosion and although light was good at deeper depths, we suspect that plants were restricted to these depositional areas which did not occur at deeper depths. The low organic substrate was also very low in ammonium. Our observations in July 1996 and comments from persons who examined the site in prior years suggest that milfoil densities were high despite the poor soil conditions. However, the poor soil may have contributed to a greater effect of weevils on plant density than would occur at other sites. It should be noted that although this is a designated trout lake, in September oxygen near the thermocline (5m, 19.4 ˚C) was < 3 mg/L and at 6m (14.1 ˚C) was < 2 mg/L.

Table 7. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1996 at Cenaiko Lake. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 2 and 3 (n=9). Secchi depth (SD), chlorophyll a (Chl-a; pooled surface and SD sample) and light and temperature profiles were taken in deep water > 100 m from the plant bed. Temperature is at 1m depth and 10% PAR depth is the depth at which light intensity was 10% of surface light (presented as the range which encompassed the 10% value).

Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/ml)	SD (m)	Temp (˚C 1m)	10% PAR Depth (m)	Plant Limit (m)
7/22/96	1.23	0.60	1.5%	1.34	5.0	25.4	4.5-5.0	3.4
2se	0.22	0.54	0.5%					
9/5/96	1.22	0.67	2.4%	5.61	4.0	25.7	5.0	3.4
2se	0.23	0.40	1.1%					

Stem and Meristem Damage

Damage was only recorded for plants from Auburn and Smith's Bay in summer 1995 due to the high effort needed to obtain these data; in 1996 few milfoil plants occurred in Otter Lake and only several whole plants were sampled. Stem damage was lowest in early summer and increased in the summer; damage in August and September ranged from 1.5% to 3.8% (Table 8). Damage at Lake Auburn was slightly higher in June and August 1995 than similar dates in 1994, however, damage was lower in 1996 than in the previous years. Damage in spring and fall at Smith's Bay in 1995 was similar to 1994, but in damage in August was higher in 1995 than 1994. The higher stem damage values at Cedar and Otter were due primarily to chironomids. Meristem damage (% of meristems missing or damaged) was considerably higher than stem damage, and typically ranged from 20-60%. Interestingly, there is no obvious relationship between stem or meristem damage and larval weevil densities.

Table 8. Mean percent stem damage (% length damaged ± 1SE) and meristem damage (% missing meristems) at the four permanent transect sites. The number of samples (n) is the number of samples for which damage was estimated. In 1996 there was very little milfoil in Otter Lake and thus not enough material or samples to estimate damage. Stem damage at Cenaiko Lake was 4.2 ±0.1% (n=17) in July and 1.9 ±0.5% (n=10) in September 1996 and 0 ± 0 and 5.5 ±2.2% in July (n=3) and September (n=6) 1997. Meristem damage at Cenaiko Lake was 62.2 ±4.0% and 54.0 ±7.2% in July and September 1996, and 22.2 ±22.2% and 39.9 ±4.9% in July and September 1997.

Stem damage

Sampling Date	Auburn	n	Smith's	n	Cedar	n	Otter	n
5/19-6/3/94	1.80 ± 0.13	10	0.44 ± 0.10	13	0.55 ± 0.21	12	2.03 ± 0.27	21
7/1-7/11/94	3.30 ± 0.59	16	1.11 ± 0.22	18	2.71 ± 0.95	14	2.91 ± 0.30	27
8/12-8/19/94	3.36 ± 0.41	15	1.88 ± 0.46	15	4.08 ± 0.85	11	2.89 ± 0.63	14
9/14-9/21/94	2.41 ± 0.41	19	1.51 ± 0.43	15	6.12 ± 1.47	19	2.26 ± 0.55	9
6/7/95-6/27/95	4.08 ± 0.49	21	0.76 ± 0.18	15	3.72 ± 0.42	13	3.08 ± 0.58	26
7/31-8/15/95	3.84 ± 1.27	15	2.79 ± 0.74	10	3.21 ± 3.21	3	-	
9/18-9/29/95	1.46 ± 0.31	11	1.51 ± 0.51	15	-		-	
6/12-6/24/96	1.77 ± 0.20	18	1.00 ± 0.46	12	1.41 ± 0.41	10	4.33 ± 2.33	2
7/30-8/9/96	2.08 ± 0.63	9	1.78 ± 0.52	9	5.11 ± 0.93	7		
9/12-9/19/96	2.93 ± 0.31	8	1.19 ± 0.30	9	3.48 ± 0.59	7		

Meristem damage

Sampling Date	Auburn	n	Smith's	n	Cedar	n	Otter	n
6/7/95-6/27/95	51.5 ± 3.9	21	40.6 ± 5.1	15	26.1± 6.0	13	-	
7/31-8/15/95	43.4 ± 6.0	15	42.5 ± 6.1	10	53.1± 15.8	3	-	
9/18-9/29/95	56.7 ± 4.7	11	41.7 ± 6.7	15	-		-	
6/12-6/24/96	36.6 ± 4.28	18	53.0 ± 6.5	12	3.1 ± 1.9	10	58.3 ± 8.3	2
7/30-8/9/96	14.6 ± 2.71	9	32.7 ± 9.2	9	21.1 ± 4.6	7		
9/12-9/19/96	29.1 ± 4.09	8	23.0 ± 6.2	9	22.4 ± 7.0	7		

Carbohydrates

Carbohydrates in milfoil at the four permanent transect sites were highest in the stems (12-22% dry mass as TNC), followed by roots and then leaves (Table 9). Stem and leaf carbohydrates were dominated by starch (> 80% of TNC as starch) whereas roots had relatively higher levels of sugars (33-60% of TNC). Carbohydrates were highest in plants from Lake Auburn and Smiths Bay; Cedar and Otter had the lowest concentrations of carbohydrates.

Table 9. Percent (of dry mass) sugar (TS), starch (Starch) and total nonstructural carbohydrates (TNC) ± 1SE for roots, stems and leaves at the four permanent transect sites in 1995-1996. Samples were collected from shallow, moderate and deep stations along transects 1, 3 and 5 (n= 5-9 at each site and date).

Response	Date	Auburn	Cedar	Otter	Smiths
ROOTS					
%TNC	6/95	9.97 ± 1.01%	6.37± 1.33%	6.18 ± 1.02%	12.73 ± 1.69%
	8/95	16.42 ± 2.10%	7.14± 1.19%	9.14 ± 0.85%	8.67 ± 0.67%
	9/95	13.88 ± 1.39%	1.89± 0.33%	4.18 ± 0.88%	9.24 ± 0.51%
	6/96	11.02 ± 0.82%	2.49 ± 0.50%	2.40 ± 0.45%	6.71 ± 1.11%
	8/96	10.95 ± 0.87%	8.78 ± 1.38%	. .	10.44 ± 1.03%
	9/96	14.30 ± 0.93%	6.71 ± 1.26%	2.74 ± 0.46%	10.83 ± 0.52%
% TS	6/95	3.43 ± 0.50%	2.52 ± 0.82%	1.96 ± 0.32%	4.03 ± 1.41%
	8/95	9.99 ± 0.73%	2.90 ±0.63%	3.97 ± 1.04%	4.29 ± 0.48%
	9/95	7.99 ± 1.14%	0.55 ± 0.12%	1.37 ± 0.33%	3.82 ± 0.28%
	6/96	5.02 ± 0.60%	1.88 ± 0.98%	1.54 ± 0.36%	1.99 ± 0.29%
	8/96	5.50 ± 0.92%	2.27 ± 0.38%	2.75 ± .	3.30 ± 0.33%
	9/96	4.11 ± 0.90%	1.48 ± 0.32%	0.55 ± 0.10%	2.30 ± 0.26%
% Starch	6/95	6.39 ± 0.70%	3.53± 1.08%	4.96 ± 0.92%	8.96 ± 2.60%
	8/95	8.01 ± 1.19%	4.78± 1.20%	5.17 ± 0.89%	4.38 ± 0.60%
	9/95	5.89 ± 1.03%	1.48 ± 0.36%	2.96 ± 0.60%	5.42 ± 0.44%
	6/96	6.00 ± 0.67%	. .	0.78 ± 0.10%	4.72 ± 0.88%
	8/96	4.88 ± 0.78%	6.51 ± 1.10%	. .	7.14 ± 0.79%
	9/96	10.19 ± 0.46%	5.14 ± 0.86%	2.18 ± 0.31%	8.54 ± 0.35%
STEMS					
%TNC	6/95	19.30 ± 1.52%	12.26± 1.72%	16.86 ± 2.17%	24.37 ± 1.73%
	8/95	21.86 ± 2.06%	11.87± 1.52%	18.43 ± 2.32%	19.32 ± 2.24%
	9/95	25.87 ± 1.57%	2.56 ±0.28%	7.96 ± 0.52%	20.20 ± 1.53%
	6/96	16.27 ± 1.06%	2.45 ± 0.81%	7.13 ± 1.64%	14.73 ± 1.01%
	8/96	18.27 ± 0.87%	14.63 ± 2.05%	4.66 ± .	25.13 ± 2.83%
	9/96	22.96 ± 1.77%	13.25 ± 2.73%	2.97 ± 0.63%	17.22 ± 1.24%
% TS	6/95	1.50 ± 0.29%	0.68± 0.19%	0.93 ± 0.26%	2.80 ± 0.54%
	8/95	4.37 ± 1.10%	2.91± 0.69%	2.22 ± 0.44%	1.73 ± 0.24%
	9/95	4.47 ± 0.48%	0.47 ±0.06%	1.51 ± 0.19%	3.14 ± 0.35%
	6/96	3.35 ± 0.31%	2.20 ± 1.81%	2.65 ± 0.35%	1.33 ± 0.18%
	8/96	3.31 ± 0.30%	0.77 ± 0.04%	0.72 ± 0.00%	3.22 ± 0.37%
	9/96	2.08 ± 0.22%	0.53 ± 0.10%	0.39 ± 0.00%	0.91 ± 0.19%
% Starch	6/95	16.74 ± 1.07%	11.62± 1.96%	15.93 ± 1.95%	21.57 ± 2.27%
	8/95	17.49 ± 2.96%	8.96± 1.86%	16.21 ± 1.94%	17.60 ± 2.36%
	9/95	21.39 ± 1.65%	2.09 ±0.33%	6.46 ± 0.34%	17.06 ± 1.58%
	6/96	12.92 ± 1.24%	. .	5.36 ± 1.30%	13.40 ± 0.99%
	8/96	14.96 ± 0.84%	13.86 ± 2.04%	3.94 ± .	21.90 ± 2.70%
	9/96	20.88 ± 1.67%	12.70 ± 2.66%	2.58 ± 0.63%	16.31 ± 1.15%

LEAVES					
%TNC	6/95	5.28 ± 0.72%	2.76± 0.32%	7.73 ± 0.58%	7.33 ± 1.13%
	8/95	9.20 ± 0.90%	5.56± 0.33%	6.57 ± 0.71%	8.72 ± 0.86%
	9/95	7.40 ± 0.25%	1.91 ±0.29%	2.01 ± 0.11%	7.94 ± 0.55%
	6/96	3.56 ± 0.19%	1.12 ± 0.05%	2.52 ± 0.36%	4.77 ± 1.85%
	8/96	4.55 ± 0.71%	6.90 ± 1.47%	2.31 ± .	10.58 ± 0.97%
	9/96	6.57 ± 0.68%	6.23 ± 1.31%	2.12 ± 0.16%	6.00 ± 1.02%
% TS	6/95	0.63 ± 0.06%	0.78± 0.30%	0.54 ± 0.01%	0.63 ± 0.13%
	8/95	0.82 ± 0.15%	0.46± 0.04%	0.49 ± 0.07%	0.39 ± 0.01%
	9/95	0.71 ± 0.09%	0.43 ±0.08%	0.40 ± 0.01%	0.54 ± 0.07%
	6/96	0.53 ± 0.05%	0.39 ± .	0.39 ± 0.00%	0.43 ± 0.04%
	8/96	0.52 ± 0.06%	0.39 ± 0.00%	0.39 ± 0.00%	0.71 ± 0.07%
	9/96	0.63 ± 0.08%	0.39 ± 0.00%	0.39 ± 0.00%	0.39 ± 0.00%
% Starch	6/95	5.69 ± 0.64%	2.54± 0.60%	6.44 ± 1.57%	5.69 ± 1.74%
	8/95	8.38 ± 0.97%	5.10± 0.34%	6.23 ± 0.78%	8.33 ± 0.86%
	9/95	6.70 ± 0.29%	1.48 ±0.33%	1.79 ± 0.11%	7.41 ± 0.60%
	6/96	3.03 ± 0.22%	. .	2.51 ± 0.97%	4.35 ± 1.82%
	8/96	4.03 ± 0.72%	6.51 ± 1.47%	1.92 ± .	9.88 ± 0.94%
	9/96	5.94 ± 0.67%	5.84 ± 1.31%	1.73 ± 0.16%	5.61 ± 1.02%

In 1995, stem, root and leaf carbohydrates were similar or increased over the summer at Auburn and Smiths Bay, but dropped significantly in Cedar and Otter in September. The low root and stem carbohydrates at both Cedar and Otter Lakes suggested that plants in these populations may have entered the winter in a low carbohydrate condition which would have reduced rates of regrowth in the following spring. In fact, root and stem carbohydrates were low in these lakes in June 1996 and June milfoil biomass was low in Cedar Lake and very low in Otter Lake, further suggesting a slow recovery due to low overwinter carbohydrate stores. The low levels in Otter Lake milfoil may help explain why the winter dieoff in spring 1996 was so severe, however, levels were even lower in Cedar Lake milfoil so it is likely that additional stress (such as severe O_2 depletion) was required to adversely affect the milfoil. Furthermore, carbohydrate levels at the Otter weevil introduction site were higher than our transect site in fall 1995, suggesting that carbohydrate levels cannot completely explain the decline. July 1996 carbohydrate levels Table 10) in Cenaiko milfoil were similar to other lakes, but September levels were quite low, suggesting that this milfoil also was ill prepared to overwinter, which coincided with a very low biomass in 1997.

Table 10. Percent (of dry mass) sugar (TS), starch (Starch) and total nonstructural carbohydrates (TNC) ± 1SE for roots, stems and leaves at Cenaiko Lake in 1996. n= 6-9 in July 1996. In September, only 3 samples with usable material were collected; few roots were obtained.

Tissue	Date	%TNC	%TS	%Starch
Roots	Jul-96	8.25% ±1.22%	0.86% ± 0.20%	7.39% ± 1.10%
	Sep-96	- .	0.39% ± .	- .
Stems	Jul-96	24.16% ± 3.93%	0.83% ± 0.12%	23.33% ± 3.96%
	Sep-96	1.63% ± .	0.39% ± 0.00%	1.24%
Leaves	Jul-96	6.48% ± 1.26%	0.39% ± 0.00%	6.96% ± 1.12%
	Sep-96	3.90% .	0.39% ± 0.00%	3.51% .

Survey sites:

Eurasian watermilfoil biomass at the four survey sites ranged from 567 g/m^2 at Gray's Bay to 3253 g/m^2 at Fish Lake (Table 11). In 1995, Eurasian watermilfoil dominant plant at all four sites. Milfoil increased from 1995 to 1996, at Fish Lake and Lake-of-the-Isles, the latter likely being due to good water clarity (Secchi Disk readings were 2.5m in July and 1.1m in August). Coontail also increased at Lake-of-the-Isles in 1996 (Appendix 3). The milfoil density at Gray's Bay and Shady Island was much lower than 1995, however, the density at Shady Island was similar to that of 1993 (1139 g/m^2). The large decrease in Gray's Bay was accompanied by a big increase in the proportion of native plants (69%; see Appendix 3) and total plant mass at both Gray's Bay and Shady Island was similar to 1995 (Table 11).

Weevils were seen at all four sites in 1996, but were only detectible in our samples at two sites: Fish Lake and Shady Island (Table 12). Weevils were seen at Gray's Bay in both 1995 and 1996, but 1996 was the first time we have found weevils at Lake-of-the-Isles since we started studying it in 1992.

Table 11. Total plant and milfoil biomass (g wet/m^2) and mean percent of plant biomass that was Eurasian watermilfoil at the four survey sites in summer 1995 and 1996. N= 9 samples (0.1m^2 quadrats) at all sites.

Lake	Date	Total Plant Biomass (g/m^2)	Milfoil Biomass (g/m^2)	% Milfoil (of biomass)	Secchi Depth (m)
Fish	9/8/95	3958	2646	60.3%	1.9
	SE	733	691	12.6%	
	9/9/96	4584	3253	79.7%	2.9
	SE	791	746	11.1%	
Gray's Bay	8/30/95	2716	2534	94.4%	2.0
	SE	718	690	3.5%	
	9/4/96	2769	567	31.3%	1.9
	SE	777	255	12.9%	
Shady Island	9/12/95	2587	2144	83.0%	1.8
	SE	309	285	5.1%	
	9/4/96	2626	1490	71.7%	2.3
	SE	591	257	10.6%	
Lake of the Isles	9/14/95	786	735	91.5%	0.5
	SE	194	218	4.3%	
	8/30/96	2022	1687	74.2%	1.1
	SE	751	751	10.0%	

Table 12. Weevil density (N/m^2 ± 2SE and number per basal stem) at the four survey sites in summer 1995 and 1996. N= 9 samples (0.1m^2 quadrats) at all sites and dates. Weevils were seen at Fish, Gray’s and Shady Island in 1995 but were not abundant enough to be detected in our samples. Weevils were seen at all four sites in 1996, and weevils were abundant enough to be found in the Isles Introduction samples in a different part of the lake. No lepidopterans were found in any samples.

Lake	Date	Larvae (N/m^2)	Pupae (N/m^2)	Adults (N/m^2)	Total (N/m^2)
Fish	9/8/95	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	per stem	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	9/9/96	1.11± 2.22	2.22± 4.44	5.56± 4.84	8.89± 10.77
	per stem	0.004±0.007	0.007±0.015	0.020±0.016	0.031±0.035
Gray's Bay	8/30/95	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	per stem	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	9/4/96	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	per stem	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Shady Island	9/12/95	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	per stem	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	9/4/96	1.11± 2.22	2.22± 4.44	3.33± 4.71	6.67± 8.82
		0.009± 0.018	0.000± 0.000	0.009±0.018	0.018±0.023
Lake-of-the Isles	9/14/95	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	per stem	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	8/30/96	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	per stem	0 ± 0	0 ± 0	0 ± 0	0 ± 0

The lower milfoil densities with increasing native plants at Shady Island and Gray’s Bay in 1996 were accompanied by lower sediment ammonium concentrations relative to 1995 (Table 13). Fish Lake and Lake-of-the-Isles, had similar sediment ammonium concentrations in 1996 relative to 1995 even though milfoil biomass increased substantially in both lakes. The large increase in percent organics and decrease in bulk density at Lake-of-the-Isles in 1996 was because in 1995 plants were restricted to very shallow water (<0.5 m) with rocky substrate, due to poor water clarity. In 1996, with better water clarity, the plants extended to deeper waters and more typical milfoil sediments.

Carbohydrate levels in plants at Fish Lake in both years (Table 14) were similar to the less stressed permanent transect sites (Table 9). Root carbohydrates were relatively low at Gray’s Bay in both years, but stem levels were were not depressed. Root carbohydrate levels were quite low at Shady Island in 1995 and both years in Lake-of-the-Isles. The low root and stem carbohydrates at Shady Island in 1995 may explain the lower density of milfoil there in 1996, however, low carbohydrate stocks at Lake-of-the-Isles in 1995 did not preclude an increase in milfoil density in 1996. In 1995, the plants at Lake-of-the-Isles were few and in poor condition, which likely explains their low carbohydrate levels. Given that densities of milfoil increased greatly in Lake-of-the-Isles in 1996, low carbohydrate reserves alone do not preclude recovery in the following year. However, the low root carbohydrates at several of these sites suggests that the milfoil is stressed and may require a significant portion of the summer to increase densities. The source of the plants in deeper water in 1996 is unknown, because milfoil was not present in water deeper than 1 m since 1993. Surveys early in the growing season would be required to determine if the plants are colonizing from seedlings, old root crowns, fragments or simply an offshore extension of the plants in shallow water.

Table 13. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium concentrations) and water column characteristics in 1995 and 1996 at the four survey sites. Sediment samples at Fish Lake were collected from shallow, moderate and deep stations along transects 1, 3 and 5 (n=9). Three samples from the intermediate depth were collected at the other survey sites. Secchi depth (SD), chlorophyll a (Chl-a; pooled surface and SD sample) and light and temperature profiles were taken in deep water > 100 m from the plant bed. Temperature is at 1m depth and 10% PAR depth is the depth at which light intensity was 10% of surface light (presented as the range which encompassed the 10% value).

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/ml)	SD (m)	Temp (˚C 1m)	10% PAR Depth (m)	Plant Limit (m)
Grays Bay								
8/30/95	0.10	6.75	34.1	6.1	2.0	25.2	3.0-3.5	3.5
2se	0.04	3.39	4.3					
9/4/96	0.12	3.29	21.3	2.1	1.9	26.2	3.0-3.5	3.5
2se	0.04	1.82	1.0					
Fish Lake								
9/8/95	0.18	4.14	28.6	7.2	1.9	23.5	2.5-3.0	4.2
2se	0.13	1.32	7.6					
9/10/96	0.08	4.30	31.7	0.3	2.9	24.1	3.5-4.0	4.2
2se	0.04	2.59	3.7					
Shady Island								
9/12/95	0.14	3.74	23.9	8.8	1.8	21.0	2.0-2.5	4.5
2se	0.05	3.12	2.8					
9/4/96	0.42	1.44	10.1	7.5	2.3	25.1	3.0-3.5	3.5
2se	0.41	0.48	9.0					
Lake of the Isles								
9/14/95	1.45	5.21	1.8	57.4	0.5	20.3	0.5-1.0	0.5
2se	0.36	4.36	1.1					
8/30/96	0.28	9.30	10.0	6.9	1.1	24.6	1.5-2.0	2.0
2se	0.08	5.32	6.7					

Table 14. Percent (of dry mass) sugar (TS), starch (Starch) and total nonstructural carbohydrates (TNC) ± 1SE for roots, stems and leaves at Fish Lake (N = 3-9), Gray’s Bay (N = 3), Shady Island (N=3) and Lake of the Isles (N=1-3) 1995 and 1996.

Lake	Date		Roots	Stems	Leaves
Fish	9/8/95	%TNC	6.09% ±0.72%	21.81% ±0.57%	6.99% ±0.29%
		%TS	2.72% ±0.94%	2.05% ±0.34%	1.27% ±0.52%
		%Starch	3.91% ±0.52%	19.93% ±0.81%	5.72% ±0.58%
Fish	9/9/96	%TNC	10.51% ±1.44%	25.42% ±1.71%	7.60% ±1.28%
		%TS	2.88% ±0.71%	2.73% ±0.85%	0.39% ±0.00%
		%Starch	7.62% ±1.06%	22.69% ±2.56%	7.21% ±1.28%
Gray's Bay	8/30/95	%TNC	5.31% ±0.16%	15.89% ±2.53%	8.76% ± 0.81%
		%TS	-	12.30% ± 4.84%	3.34% ± 0.12%
		%Starch	-	5.84% ± 2.84%	5.41% ± 0.76%
Gray'sBay	9/4/96	%TNC	4.52% ±0.27%	18.40% ±0.41%	5.80% ±1.88%
		%TS	2.21% ±0.23%	1.96% ±0.31%	0.48% ±0.09%
		%Starch	2.39% ±0.11%	16.44% ±0.52%	5.32% ±1.79%
Shady Island	9/12/95	%TNC	2.95% ± 0.74%	8.99% ± 5.69%	1.72% ± 0.28%
		%TS	1.69% ± 0.58%	0.79% ± 0.28%	0.39% ± 0.04%
		%Starch	1.82% ± 1.26%	8.20% ± 5.84%	1.33% ± 0.28%
Shady Island	9/4/96	%TNC	8.80% ±0.43%	22.39% ±3.01%	10.32% ±0.32%
		%TS	2.78% ±0.08%	6.14% ±1.51%	0.75% ±0.24%
		%Starch	6.02% ±0.51%	16.26% ±1.93%	9.57% ±0.30%
Lake of the Isles	9/14/95	%TNC	2.24% ± -	2.18% ± -	1.13% ± -
		%TS	0.81% ± -	0.89% ± -	0.38% ± -
		%Starch	1.43% ± -	1.28% ± -	0.76% ± -
Lake of the Isles	8/30/96	%TNC	2.87% ±0.32%	15.17% ±1.86%	9.38% ±0.11%
		%TS	0.39% ±0.00%	0.39% ±0.00%	0.39% ±0.00%
		%Starch	2.48% ±0.32%	14.78% ±1.86%	8.99% ±0.11%

Weevil Introduction/Manipulation:

Otter Lake 1995:

There were no significant differences in sediment characteristics (Table 15) or in root, stem or leaf carbohydrates (Table 16), between control and stocking plots prior to manipulation or after manipulation, however, sediment ammonia appeared to decline less in the stocked plots than in the control plots (Table 15). The high bulk density values and variances in the control plots was due to one anomalous plot; when those data removed the bulk densities were quite similar between treatments. Counts at one week after stocking suggested that the stocking was successful; adults were found in most of the stocked plots whereas no eggs and fewer adults were found in the control plots (Table 17). Damage was somewhat higher in the stocked plots. However given the short exposure time and low counts these data indicate no significant differences and are only suggestive. Inclement weather precluded additional visual counts.

Table 15. Sediment characteristics at the weevil augmentation site in Otter Lake before and four weeks after weevils were stocked (100 adults and 45 larvae per 12m^2 plot). N=5 replicates for each treatment and date.

Session	Date	Weevils Stocked	Bulk wt (g/ml)	NH_4 mg/L	%N TKN	% Organic
Pre	8/29/95	0	0.18	4.0	2.2	41.2%
	2se		0.20	1.5	0.9	17.2%
	8/29/95	100	0.08	4.3	2.4	44.6%
	2se		0.02	2.4	0.2	4.7%
Post	10/2/95	0	0.28	1.5	2.2	43.2%
	2se		0.43	0.9	1.0	21.0%
	10/2/95	100	0.08	2.5	2.2	45.7%
	2se		0.02	1.7	0.3	5.0%

Table 16. Plant carbohydrate levels (%TNC) at the weevil augmentation site in Otter Lake before and four weeks after weevils were stocked (100 adults and 45 larvae per 12m^2 plot). N= 2-6 replicates for each treatment and date. No differences among weevil stocking density were found for %TNC, %TS or %Starch on either date.

Session	Date	Weevils Stocked	%TNC Roots	%TNC Stems	%TNC Leaves
Pre	8/29/95	0	8.76%	29.17%	9.19%
	2se		1.33%	0.15%	2.15%
	8/29/95	100	8.16%	32.14%	9.79%
	2se		2.72%	7.64%	2.35%
Post	10/2/95	0	13.18%	15.83%	6.64%
	2se		3.51%	3.46%	0.87%
	10/2/95	100	10.71%	17.92%	5.66%
	2se		5.19%	4.65%	1.91%

Table 17. Mean counts of weevil eggs, adults and percent of stems counted that were damaged in the control and weevil treatment plots at Otter Lake one week after 145 weevils were stocked. N=5 for each treatment.

Session	Date	Weevils Stocked	No. eggs	No. adults	%Stems Damaged
	9/13/95	0	0.0	0.4	7.7%
	2se		0.0	0.5	5.7%
	9/13/95	100	0.2	1.0	11.3%
	2se		0.4	1.1	4.1%

Milfoil densities in the plots were high. Initial (pre-stocking) plant biomass averaged 6278 g/m^2 of which 5088 g/m^2 was Eurasian watermilfoil. Four weeks after stocking, milfoil biomass averaged 5187 g/m^2 in control plots and 4597 g/m^2 in stocked plots (Table 18). There was no difference in milfoil density between treatment and control plots before or 4 weeks after stocking and there were no differences in milfoil density within plots before or after stocking (all $P \geq 0.5$). Interestingly, no weevils were found in quantitative samples in the treatment plots before or after stocking (Table 18), however a low density of weevils was present in the control plots both before (0.7/m^2) and after (1.3/m^2) stocking. The extreme cold weather that occurred during the second and third weeks after introduction and the late initiation of stocking probably precluded finding any major differences associated with stocking. Our efforts do suggest that stocking plots is feasible and that weevils will establish within these plots; plot stocking will be carried out in late spring 1996 with the stock of diapaused weevils we have refrigerated.

Table 18. Plant and weevil densities observed at the weevil augmentation site in Otter Lake before and four weeks after weevils were stocked (100 adults and 45 larvae per 12m^2 plot). N= 15 replicates (0.1m^2 quadrats) for each treatment and date. No significant differences in milfoil biomass or weevil density were found with weevil stocking density (all $P \geq 0.5$).

Otter intro data Session	Date	Weevils Stocked	Milfoil Biomass (g wet/m^2)	Non-Milfoil Biomass (g/m^2)	Weevil Density N/m^2
Pre	8/29/95	0	5288	796	0.7
	1se		644	275	0.7
	8/29/95	100	5199	1158	0.0
	1se		581	176	0.0
Post	10/2/95	0	5187	576	1.3
	1se		447	130	0.9
	10/2/95	100	4597	317	0.0
	1se		714	80	0.0

Cedar Lake and Lake of the Isles 1996:

Counts along the transects suggest that stocking was not effective at either increasing adult weevil density, eggs or stem damage (Table 19). Very few weevils were spotted (only one or two per session in each treatment) and after week 2 no eggs were sighted. Damage was low and no persistent pattern was noted. Milfoil biomass increased in the control plots and decreased in the treatment plots (Table 20), however, there were no significant differences between the plots before or after stocking. Given that final weevil density was higher (but not significantly) in the control plots than in the treatment plots, it is unlikely that weevils resulted in the changes in plots. All life stages were present, but adults were more common than other stages. It should be noted that weevils were found in both Cedar Lake and Lake of the Isles and 1996 is the first year of documented occurrence of weevils in Lake-of-the-Isles.

Table 19. Mean counts (15 min along 10m transect) of weevil eggs, adults and percent of stems counted that were damaged in the control and weevil treatment plots at Cedar and Lake-of-the-Isles 2, 5 and 7 weeks after weevils were stocked. N=7 for each treatment.

Session	Date	Weevils Stocked	No. eggs	No. adults	%Stems Damaged
2 weeks	7/23/96	0	0.57	0.14	7.1%
	2se		0.74	0.29	4.3%
		100	0.86	0.00	7.6%
	2se		0.92	0.00	5.2%
5 weeks	8/13/96	0	0.00	0.14	1.0%
	2se		0.00	0.29	1.1%
		100	0.00	0.29	1.7%
	2se		0.00	0.57	1.6%
7 weeks	8/27/96	0	0.00	0.14	1.3%
	2se		0.00	0.29	1.3%
		100	0.00	0.00	0.9%
			0.00	0.00	1.4%

Table 20. Plant and weevil densities observed at the weevil augmentation site at Cedar and Lake-of-the-Isles before and 2 months after weevils were stocked (100 adults per 12m^2 plot). N= 14 replicates (0.1m^2 quadrats) for each treatment and date. [1]= predominately adults, [2] = equal numbers of adults, larvae and pupae.

Session	Date	Weevils Stocked	Milfoil Biomass (g wet/m^2)	Non-Milfoil Biomass (g/m^2)	Weevil Density N/m^2
Pre	7/12/96	0	2128	1503	0.71[1]
	2SE		827	1232	1.43
	7/12/96	100	3490	1161	0
	2SE		1232	1203	0
Post	9/24/96	0	3031	497	7.86[2]
	2SE		971	323	7.61
	9/24/96	100	2420	442	3.57[1]
	2SE		1031	348	5.78

Overwinter assessments:

Collection of Adult Weevils for Augmentative Release: Weevils collected from soil samples have significantly higher survival (81%) than those collected from the water (9.5%; Table 21). From these results it appears that weevils collected in late fall from the water are not able to survive at 4 ˚C . Adults collected from water may not be physiologically able to survive an extended period of cold temperatures or they need additional "conditioning" once returned to the lab to induce diapause. Our preferred method of collecting and storing weevils for augmentative release is to collect insects from soil samples as late as possible in the fall (late October are early November) and hold weevils at 4 ˚C until needed the following spring.

Table 21. Survival of adult *Euhrychiopsis lecontei* on 3 April 1996 of weevils collected from soil samples (Smith's Bay - November 1995) or milfoil shoot tips (Lake Auburn, October-September, 1995) and held at 4 ˚C.

Source	**Date Placed at 4°C (1995)**	**Initial Number**	**Number Alive 3 April 1996**	**% Survival**
Smith's Bay (soil)	14-Nov	50	42	84
"	6-Nov	55	43	78
			Average Survival	81
Lake Auburn (water)	17-Oct	25	1	4
"	11-Oct	25	0	0
"	2-Oct	25	5	20
"	30-Sep	25	0	0
"	10-Oct	25	0	0
"	10-Oct	25	0	0
"	25-Oct	25	5	20
"	10-Oct	25	8	32
			Average Survival	9.5

Adult Dispersal. Pitfall sampling, is not an efficient method of measuring weevil activity. Only 11 traps out of 195 contained weevils. This type of sampling was discontinued in 1995. No weevils were collected from window pane traps at Lake Auburn from either spring or fall sampling. A single weevil was collected at Smith’s Bay on the water side of a vertical trap in the fall. This sampling method is not able to capture weevils in flight. Either weevils are not flying or they are dispersing at greater heights than 4 feet. Passive trapping was discontinued and an attractive yellow trap was used in subsequent years. These modified Japanese beetle traps captured only a few individuals. So few weevils were captured in the modified yellow Japanese beetle traps (Table 22) that meaningful conclusions are not possible. Our observations are that weevils do take wing in the Spring as evidenced by the few adults we captured. However, at the same time most weevils have left the overwintering site and are readily found along the shoreline feeding on floating milfoil stems. It appears that few insects are flying in the spring or the yellow traps are not overly attractive to these insects.

Table 22. Total number of weevils captured by beetle trap location at both Lake Auburn and Smith's Bay sampling sites.

	Shoreline		**In-Water**	
Date	**Lake Auburn**	**Smith's Bay**	**Lake Auburn**	**Smith's Bay**
8-May	2	0	0	0
16-May	1	0	0	0
22-May	0	0	0	1
28-May	0	0	0	0
11-June	1	n/a	0	n/a
Total	4	0	0	1

Litter Sampling: Weevil population densities decrease in the Spring between mid-April and early-May when they leave the soil to enter the water, and increase in the Fall between mid-Sept. to early-Nov. as they come in from the water to colonize the terrestrial overwintering site (Table 23).

Mean weevil numbers for Spring and Fall seasons at Lake Auburn were compared to calculate a percent mortality for the 1993-6 years. Weevil numbers declined in soil samples by 77.9% in 1993-4 and 53.6% in 1995-6 over the winter. Weevil density in 1994-was too low to accurately determine winter mortality. The mean percent mortality was 65.7% for both 1993-4 and 1995-6 seasons.

Data shown in Fig. 4 are those dates where maximum number of weevils are collected and from samples of similar type so that comparisons between years and sites can be made. Lake Auburn weevil numbers in Fall 1993 soil samples were 25-fold higher than weevil density found in Fall 1994 and soil abundance was low in spring 1995. Weevil numbers increased at both sites in 1995 and 1996. In fall 1996, average density of weevils at Lake Auburn was 20.8/sample and 42.7/sample at Smith’s Bay. Litter samples were again taken Spring and Fall, 1997, specifically (21 April and 29 October, 1997). In the spring samples an average of 21.4 weevils were found per sample at Lake Auburn, and 68.25 at Smith’s Bay. These weevil densities are the highest densities yet observed in the four years of litter sampling. Winter mortality was the lowest observed as well. Snow cover may be an important prerequisite for good survival. Sample variance is large at Smith’s Bay (Fig. 4) because of the limited amount of area available to us to collect litter samples.

Density of weevils was low at Cenaiko lake in both Fall 1996 and Spring 1997 (Table 23) relative to the other sites. In part, this may be due to the decline in weevil abundance in summer 1996 associated with the decline in milfoil.

Table 23. 1996 Spring and Fall soil samples taken at Lake Auburn; Smith's Bay and Cenaiko Lake sites. Density in Spring 1997 is presented for Cenaiko Lake; 1997 data for Lake Auburn and Smith's Bay are presented in Figure 4.

Date	**Site**	**# Sampled**	**Total # of Weevils**	**Mean # of Weevils per $0.20 m^2$ Sample**
April 17	Lake Auburn- Spring	8	31	3.9
May 1	"	4	9	2.3
Sept. 13	Lake Auburn- Fall	6	2	0.3
Sept.24	"	6	15	2.5
Oct. 3	"	6	16	2.7
Nov. 4	"	6	125	20.8
April 17	Smith's Bay- Spring	8	87	10.9
May 1	"	4	25	6.3
Sept. 13	Smith's Bay- Fall	6	8	1.3
Sept. 24	"	6	99	16.5
Oct. 3	"	6	32	5.3
Nov. 4	"	6	256	42.7
Sept. 13	Cenaiko Lake- Fall	6	0	0
Sept. 24	"	6	33	5.5
Oct. 10	"	15	59	3.9
Nov. 12	"	6	5	0.8
April 97	Cenaiko Lake Spring	6	3	0.5

It is not clear what is controlling fall overwinter densities; no relationship is apparent with fall in-water densities (Table 24). Perhaps prevailing winds and rafting of milfoil is important to fall overwinter densities. There is some suggestion that spring shoreline litter densities are related to early summer in-lake densities, which does suggest that overwinter density and survival could have a direct effect on at least early summer in-lake weevil densities.

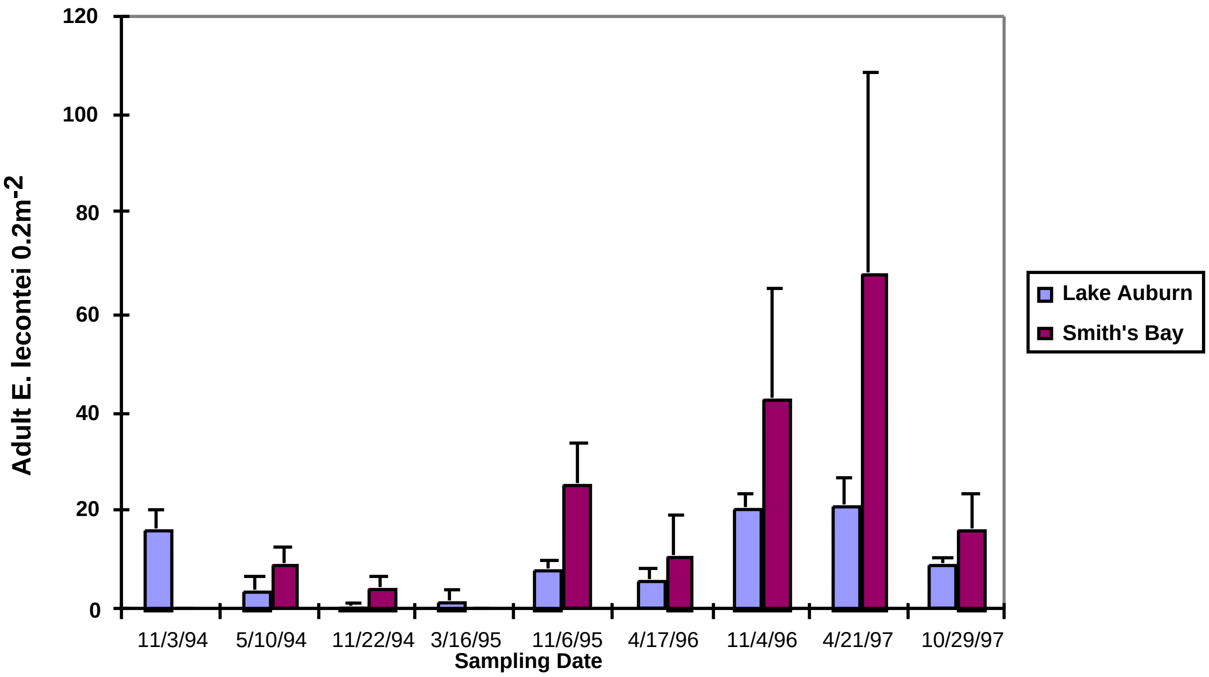

Fig. 4. Mean number of *E. lecontei* adults per 0.2 m^2 shoreline litter samples at Lake Auburn and Smith's Bay at the peak for each sampling period.

Table 24. Comparison of spring and fall in-lake weevil densities with shoreline densities at Lake Auburn and Smith's Bay. In-lake samples were the earliest and latest samples each year and shoreline densities are for peak densities (i.e., after all on shore or before they left the shore). *weevils found in water along lake edge.

	Lake Auburn		Smith's Bay	
	In-Lake Density	Shoreline Density	In-Lake Density	Shoreline Density
Date	N/m^2	N/m^2	N/m^2	N/m^2
Fall 1993	4.0	110.7	2.5	.
1SE	1.4	6.7	2.5	.
Spring 1994	35.6	19.4	4.6	46.0
1SE	18.3	4.1	3.3	16.9
Fall 1994	11.7	3.6	3.6	25.0
1SE	5.9	1.5	2.3	7.0
Spring 1995	7.7	11.3	1.2	4.25*
1SE	1.3	8.3	0.7	.
Fall 1995	9.4	42.1	3.6	127.0
1SE	1.7	8.1	2.5	41.1
Spring 1996	33.0	29.4	4.8	56.9
1SE	9.8	14.1	2.9	38.1
Fall 1996	12.0	104.0	6.0	213.5
1SE	3.3	12.5	2.7	110.3

Temperature-development time relationships:

Weevil development followed a predictable relationship with temperature (Fig. 5). Fastest development times were at 27-31 ˚C and will likely decline above 31 ˚C. Development times ranged from 60d at 15 ˚C to 17d at 27-31 ˚C (Table 25). All stages were influenced by temperature, but egg hatch showed the least increase in rate at the higher temperature. It should be noted that development was successful for about 75% of the weevils at temperatures above 15 ˚C, but only 4 weevils successfully developed at 15 ˚C and less than half the eggs hatched. Based on total development from egg to adult, the threshold development temperature is 10 ˚C (Fig. 5). These data will be useful in modelling development times and population dynamics given lake water temperatures. Based on these data and limited analysis of continuous lake temperature data, weevils should complete development in about 25 days during June to mid-September.

Table 25. Weevil development times (days) for each stage at 15-31 ˚C (C. Mazzie was instrumental in collection of these data).

Temperature	Egg Hatch	Larva to Pupa	Pupa to Adult	Total (egg-adult)
15 ˚C	13.6	18.0	28.0	58.7
19 ˚C	7.6	11.7	13.6	31.8
23 ˚C	5.8	8.4	9.9	24.1
27 ˚C	4.8	4.9	7.2	17.1
31 ˚C	5.0	5.4	7.4	17.3

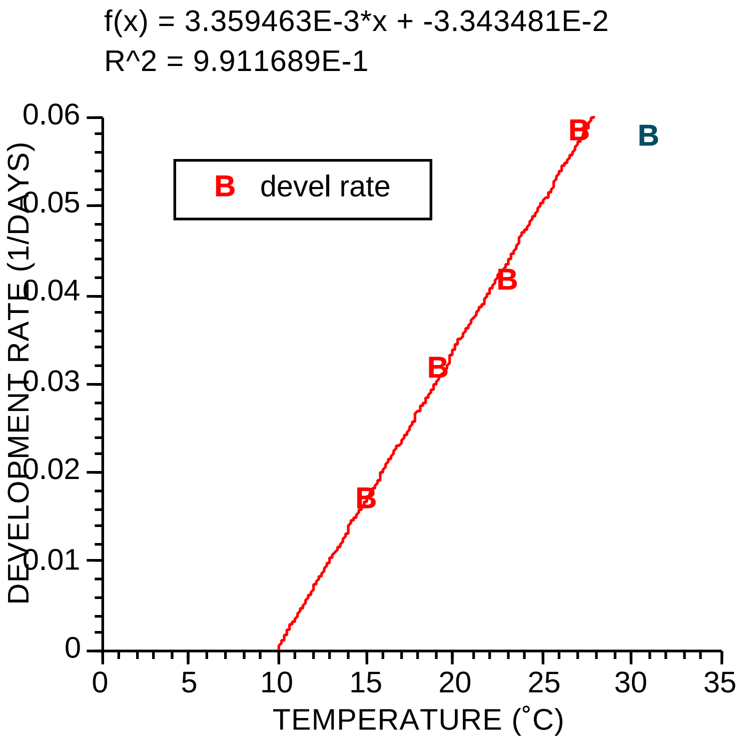

Figure 5. Developmental rate (1/days) of *E. lecontei* from egg to adult at 5 temperatures. The lower temperature threshold for development is estimated by the X intercept, 10 ˚C. The 31 ˚C point was not fit to the regression because it is clearly at or above the maximal development rate.

Genetic variation in weevil populations in relation to host-plant:

In preliminary analysis of the data on length, we found that regardless of source population, weevils reared on Eurasian watermilfoil for any amount of time (i.e. 3 of the 4 treatments) were significantly larger than weevils reared from egg to adult on northern watermilfoil ($p<0.05$). Using REML, we found a large component of V_A in length for Eurasian-reared progeny of both source populations. For Eurasian-source progeny, $V_A = 0.43$ and for northern-source progeny $V_A = 0.20$, although this is significant for the Eurasian-source progeny only ($p=0.1$). In contrast, we found that V_A was not an important component of variation in length for northern-reared progeny ($V_A =0$ and 0.007 for Eurasian- and northern-source populations respectively).

Our preliminary results suggest that Eurasian watermilfoil may promote larger adult sizes in weevils than northern does. This is consistent with our previous findings which suggested that Eurasian may be a superior hostplant because weevils tend to develop faster on Eurasian than on northern (Newman et al. 1997). The results on V_A reveal that there is little or no evolutionary potential for a response to selection on this trait (length) by weevils reared on northern. However, there may be substantial potential for adaptation by weevils reared on Eurasian watermilfoil. This analysis is currently being conducted with the hostplant preference, weight, and development time data.

Synthesis

We have now documented significant and persistent declines of Eurasian watermilfoil at 3 sites: Otter Lake, Smith's Bay on Lake Minnetonka, and Cenaiko Lake. The decline at Otter Lake was most likely due to a severe winter kill rather than the direct action of potential control agents, however, the persistence of the decline may be enhanced by damage by the weevil *E. lecontei* and perhaps also lepidopterans. The decline at Cenaiko Lake was unquestionably due to weevil damage during the summer of 1996 and the decline persisted through 1997. The slow decline at Smith's Bay has been accompanied by persistent weevil damage and moderate weevil densities and a positive response by the native plant community. These declines complement the list of declines associated with weevil occurrence compiled by Creed (*in press*), however, unlike most previous associations we have presented pre and post quantitative data on both plant biomass and weevil densities.

Eurasian watermilfoil remained at relatively low levels after a slow decline at Smith's Bay from 1994 to June 1996, and this was accompanied by an increase in the density of non-milfoil macrophytes. Plant community response appears to be important in preventing milfoil from increasing in abundance. Although the decline at Otter Lake does not appear to be directly attributable to weevils, we did find some weevils on the few remaining plants we found. It is possible that *E. lecontei* was a factor in preventing explosive regrowth of Eurasian watermilfoil over the summer. The rapid response by the native plant community may have also helped prevent a resurgence of Eurasian watermilfoil. Although milfoil in Otter Lake in the fall of 1995 appeared to have low carbohydrate stores, which could have contributed to its demise, low carbohydrates alone do not appear to be the cause of the decline because milfoil at Cedar Lake had similar low levels of carbohydrates and did not show a catastrophic decline. However, milfoil biomass at Cedar Lake in 1995 and 1996 was low (<700 g/m^2 after August 1995) and the lowest densities, in fall 1995 and spring 1996 were associated with low carbohydrate levels. The potential declines at Shady Island and Gray's Bay were both associated with relatively low root carbohydrates in 1995 and reduced ammonium in 1996, however, natural year to year variability may be great. In fact, the sites with low carbohydrates have not sustained densities of milfoil comparable to sites with consistently high levels (e.g., Fish, Auburn and Smith's, and Otter before the decline), suggesting some positive relation of individual plant health (carbohydrates) to population density. It is possible that competition with other plants can reduce sediment nutrients and result in a reduced ability to store carbohydrates and further expand, but further study is needed to relate plant health to competition and possible declines.

Cenaiko Lake clearly demonstrated the effects that high densities of weevils can have over one summer. The results (and the individual plants) looked similar to our tank experiment (Newman et al. 1996a), even though weevil densities were only around 100/m^2. However, weevil densities in June may have been even higher and due to the relatively shallow area inhabited by much of the milfoil, the effects may be greater than in Lake Auburn which reached similar weevil densities in July 1994. Weevil densities per stem, however, were much higher in Cenaiko and at 1.6 per stem were comparable to the final density of weevils in our high density treatment in the tank experiment (1.8 per stem; Newman et al. 1996a). Although total weevil density declined with the declining milfoil, September densities remained above 0.6/stem. We do not know what enabled weevil populations to attain such high densities at Cenaiko. Visual examination does not suggest superior overwinter habitat. Fall 1996 shoreline densities were lower than Auburn and Smith's Bay, however, weevils may be spread over a larger area at Cenaiko. By fall, in-lake densities at Cenaiko were much lower due than in July, due to the lack of healthy milfoil to sustain a high population, however, the in-lake densities were not much lower than either Smith's Bay or Lake Auburn, which had much higher shoreline densities. The weevil damage did appear to reduce stem carbohydrates and the depressed carbohydrate values may have limited the ability of the milfoil to recover in 1997.

A key unresolved issue is what is limiting weevil densities at our sites. Overwinter mortality does not appear extreme, however, spring in-lake densities may be determined by overwinter population levels. What determines fall shoreline densities is less clear as there is no apparent relationship with fall in-lake densities. Factors affecting summer populations appear more important overwinter success as evidenced by the failure to develop high summer populations at Otter Lake despite relatively high spring populations there in 1994 and 1995. Similarly, weevils have never maintained detectable levels at Cedar Lake after August. Laboratory work by Sheldon and O'Bryan (1996a) and our group (Newman et al. 1997; data in this report) suggests that under good conditions weevil mortality is low, and weevil populations should build rapidly during the summer; with a mean development time of 24 d at 23 ˚C, at least 3 or 4 generations should be produced at most sites. Temperatures at the surface of dense milfoil beds are closer to 30 ˚C (data from temperature recorders) so temperatures at 1m are conservative estimates of temperatures experienced by weevils. If predation or plant resistance are not limiting weevils, the populations should build rapidly over the course of the summer. Thus, overwinter density may be important at lakes with very low density, but in lakes with moderate overwinter densities, in-lake factors may be more important.

Work by Sutter and Newman (1997) suggests that sunfish predation has the potential to limit in-lake populations, particularly with low weevil densities. At moderate to high weevil densities (30-70/m^2), sunfish were predicted to have a little effect on weevils, consuming <5% of the population per day even at high fish densities. However, at low weevil densities (5/m^2), sunfish could consume substantial proportions of the weevil population (5-30%), even at moderate fish densities and predation rates. Fish predation could explain the apparent failure of the augmentation plots in Otter and Cedar/Isles. Fish predation could also limit initial weevil population build up in the spring or affect weevils getting to shore in the fall. Further investigation of sources if mortality and factors limiting weevil populations is needed.

Clearly, high densities of weevils can have direct and persistent effects on the plant. The damage at Cenaiko was substantial and obvious at densities of 100 weevils/m^2 (or >1/stem). This observation and the slow decline at Smith's Bay suggest that we may have overestimated the density of weevils needed to effect a decline; perhaps 50-100 weevils per m^2 is adequate. However, it is also becoming clear that a density at this level is no guarantee of successful control. Weevil densities in Lake Auburn in 1994 were 36 and 103 per m^2 (0.15 and 0.3 per stem) in May and July, yet the milfoil increased dramatically in 1995 when weevil densities did not exceed 10/m^2. Conversely, consistently lower weevil densities in Smith's Bay have been associated with a slow but persistent decline. Densities in shallower water, where effects are most apparent, are higher, but still not generally over 50/m^2; densities per stem at the two shallowest sites in Smith's Bay ranged from 0.06 per stem to 0.4 per stem over the two years. A key difference appears to be plant community response. Newman et al. (*in press*) noted that plant competition and environmental factors are often important to the success of biological control.

Smith's Bay has generally retained 10-11 species (3-4 per sample) and other plants have increased while Eurasian watermilfoil decreased. In Lake Auburn, after Eurasian watermilfoil declined in 1993, the number of native plant species declined, as did their contribution to plant biomass and milfoil increased. Furthermore, the native plant community was dominated by coontail, which generally composed over 90% of the non-milfoil biomass. Observations in the field suggest that good native plant response to patches opened by damage milfoil may inhibit regrowth of milfoil. In Lake Auburn, damaged and arched milfoil would simply colonized adjacent open patches. Similarly, the dense coverage of native plants in Otter Lake may be inhibiting the further expansion of the remaining Eurasian watermilfoil plants. We suspect that if open areas were not colonized by other plants, the Eurasian watermilfoil would have rebounded in 1997. Declines without a subsequent rebound in non-milfoil plants are not likely to persist. The reductions in milfoil at Shady Island and Gray's Bay were both associated with increases in native plants, suggesting that these reductions may also persist.

One factor that may affect the plant response is water clarity. Lake Auburn has typically had poor mid-summer water clarity that might inhibit growth of other plants, along with a dense milfoil canopy. In contrast, Smith's Bay retains good water clarity throughout the season, which might alow better native plant response. Prior to alum treatments, water clarity decreased markedly in mid summer in Cedar Lake, after milfoil had shaded out the native plants, and the subsequent decrease in healthy milfoil, in late summer may have prevented an increase in weevil populations. Water clarity in Otter Lake decreased after the catastrophic milfoil decline, but this appeared due to suspended organics rather than algae (compare Chlorophyll values with Secchi disk readings). Furthermore, with little Eurasian watermilfoil, the plants were not being further shaded by milfoil. One concern is the continued response of Eurasian watermilfoil in Cenaiko Lake. Although water clarity was high in 1996, clarity was relatively low in 1997, in part due to sediment runoff from high rains. The poorer water clarity may have inhibited additional expansion of some native plants such as *Chara* and it remains to be seen if the decline will persist for several years.

The improved water clarity in Cedar Lake and Lake of the Isles, associated with both favorable weather and alum treatments will likely result in at least short term increases in milfoil abundance. However, such season long increases may be needed to support weevil populations and positive plant community response; it remains to be seen if milfoil will remain dominant or if weevil populations will build and native plants can respond.

One positive development at several sites is the reappearance of northern watermilfoil. Northern watermilfoil was found in shallow water at Auburn and Otter and is fairly abundant at the shallowest station at Smith's Bay. During the increase of Eurasian watermilfoil in the early to mid 1990s, northern watermilfoil virtually disappeared from our sites. Although weevils prefer Eurasian to northern watermilfoil once they have been exposed to it (Solarz and Newman 1996), it is clear that the northern is also acting as a host for the weevil in these lakes. At Smith's Bay in particular, but also a Lake Auburn, northern watermilfoil plants were hosting weevils and showed signs of damage. These populations should be able to support weevils in the event of Eurasian watermilfoil declines. In fact, in Otter Lake, it appears that there may be as many weevils as there were before the milfoil decline. Because milfoil is so rare, we have not sampled weevils in our quadrats since the decline, however, field observations suggest that weevils occur on 25% or more of the existing Eurasian watermilfoil plants.

We have no evidence that the milfoil declines are associated with soil nutrient depletion, however, there is some suggestion again that native plants may compete for nutrients with Eurasian watermilfoil, perhaps slowing its growth response. Low root and shoot carbohydrates appear associated with stressed plants and acute declines, but at least at our level of seasonal resolution, carbohydrate levels are not clear predictors of declines; several additional factors appear important.

In summary, our work to data and that of others (e.g., Creed and Sheldon 1995, Sheldon 1997, Creed *in press*) shows that the weevil *E. lecontei* can cause significant declines of Eurasian watermilfoil. However, we cannot yet predict when, where or how with much accuracy. Several factors will be critical to our ability to predict declines, including a better understanding of what limits or regulates weevil populations, what affects milfoil response to weevil populations and what influences plant community response to weevil-stressed Eurasian watermilfoil. Field assessment of the importance of fish predation is needed. Comparisons of predicted weevil population dynamics to field observations may also enable us to identify limiting factors. A comparison of the attributes of lakes with high and low weevil populations (including overwintering conditions) might be instructive. A key factor in success will be the persistence of declines; the decline in Fish Lake (Lillie 1996) has apparently not persisted, possibly due to failure of a strong native plant community response. A better understanding of milfoil response to weevil damage and of plant community interactions will be important to being able to predict or manage persistence of declines. The role of water clarity and nutrient levels on these interactions should be investigated. A combination of field observations and manipulations and laboratory and modelling exercises will probably be needed. It is also likely that biocontrol will work in some environments and not others. The next step is to predict these environments and the management actions that will enhance, not inhibit, control.

Conclusions

- Milfoil biomass in Otter Lake crashed over the winter of 1995-1996 from 2600 g/m^2 in September 1995 to 20 g/m^2 in June 1996. The milfoil continued to decline during the summer to <1g/m^2 while native plant biomass increased from 430 g/m^2 in June to almost 2000 g/m^2 in September. This dramatic decline was likely due to anoxia associated with a winterkill, independent of weevils, however, weevils were present on the few remaining milfoil plants throughout the summer of 1996 and 1997. The decline persisted through 1997 with milfoil <1/100th of its previous densities, but the native plants increased to over 3000 g/m^2 by September 1997.

- Biomass of milfoil in Smith’s Bay continued to decline from a peak in early July 1994 to 665 g/m^2 in June 1996; it increased to 1650 g/m^2 in September, but remained less than half of the plant biomass. Milfoil further declined in 1997 to <1100 g/m^2. In 1996 milfoil did not surface in most of the Bay and remained <60% of total macrophyte biomass throughout the summer; in September 1997 milfoil was <40% of plant biomass. Non-milfoil biomass increased substantially during the 1996 and 1997 from < 900 g/m2 in 1995 to almost 1900 g/m^2 in September 1997. These are the highest densities and greatest percentages of non-milfoil plant biomass we have observed at Smith’s Bay in 5 years of study. Weevil densities were higher in 1996, but not as high as peak levels in 1994.

- A new site, Cenaiko Lake, was examined in 1996, initially as a site for augmentation. Weevil damage was extensive and weevil densities were the highest we have seen (103/m^2 in July 1996). Milfoil biomass in Cenaiko Lake decreased from 974 g/m^2 in July to 239 g/m^2 in September 1996 while native plant biomass increased from 900 g/m^2 to over 1400 g/m^2. Weevil density was much lower in September, probably due to lack of suitable milfoil. This decline persisted through 1997 with milfoil biomass ranging from 8 to 30 g/m^2, and native plants increasing to over 90% of plant biomass.

- Milfoil biomass in Lake Auburn increased over the summer in 1995 to over 5000 g/m^2; it increased in 1996 from about 3000 g/m^2 in June and August to 3600 g/m^2 in September, however, in contrast to previous years, milfoil did not exceed 70% of total plant biomass. Milfoil biomass stayed below 3000 g/m^2 in 1997. Weevil densities were higher in 1996 than in 1995, but did not reach the high densities of early 1994. Milfoil biomass in Cedar Lake showed a typical summer peak and fall decline in 1995, associated with water clarity. Biomass in 1996 hovered around 600 g/m^2, however, due to high water clarity milfoil was present at deeper stations it had previously been absent from and did not decrease in late summer as it did in previous years. Weevil densities reached detectible levels at Cedar in 1996, the first time since July 1994, but densities remained low.

- Shoreline overwinter mortality (Fall to Spring) at Lake Auburn ranged from 80% in 1993-1994 to 54% in 1995-1996. Shoreline densities in Fall 1996 and Spring 1997 at both Smith’s Bay and Lake Auburn were the highest we have seen since monitoring started in 1993 and there has been an increase in fall shoreline densities from 1994-1996. Fall shoreline densities were 33/m^2 at Cenaiko Lake, 104/m^2 at Lake Auburn and 214/m^2 at Smith’s Bay. Low shoreline densities at Cenaiko in both fall and spring may have been due to a low population after the milfoil decline. It is not clear what determines fall overwinter densities but spring in-lake densities do appear related to spring shoreline densities.

- Milfoil increased from 1995-1996 at two of our four survey sites, Fish Lake and Lake-of-the-Isles, but milfoil decreased substantially while native biomass increased at Shady Island and Gray’s Bay of Lake Minnetonka. Weevils were seen at all four sites, but the highest densities were at Fish Lake and Shady Island in 1996.

- Weevil introduction into open augmentation sites at Otter Lake in 1995 and Cedar/Isles in 1996 showed no clear indication of any significant weevil effects on milfoil and weevil densities did not increase above several per m^2. Fish predation or plant resistance are suspected to have prevented establishment of high weevil densities and equal or greater densities of weevils were seen in our control plots.

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Appendix 1. Biomass (g wet/m^2) of plants collected at the permanent transect sites and abbreviations used in this report.

Key to plant abbreviations in Appendix tables 1-3.

CHA	*Chara* spp. (muskgrass)
CRT	*Ceratophyllum demersum* (coontail)
ELD	*Elodea canadensis* (Canada waterweed)
HET	*Heteranthera dubia* (mud plantain) = *Zosterella dubia*
LMR	*Lemna minor* (lesser duckweed)
LTR	*Lemna trisulca* (star duckweed)
MGD	*Megalodonta beckii* (water marigold)
MSI	*Myriophyllum sibiricum* (northern watermilfoil)
MSP	*Myriophyllum spicatum* (Eurasian watermilfoil)
NAJ	*Najas* spp.
NMP	*Nymphaea* spp.
NUP	*Nuphar* spp.
PAM	*Potamogeton amplifolius* (largeleaf pondweed)
PBE	*Potamogeton berchtoldi* (Berchtolds' pondweed)
PCR	*Potamogeton crispus* (curled pondweed)
PDI	*Potamogeton diversifolius*
PEC	*Potamogeton pectinatus* (sage pondweed)
PFO	*Potamogeton foliosus* (leafy pondweed)
PGR	*Potamogeton gramineus* (variable pondweed)
PIL	*Potamogeton illinoensis* (Illinois pondweed)
PNA	*Potamogeton natans*(floating leaf pondweed)
PNO	*Potamogeton nodosus* (river pondweed)
PRI	*Potamogeton richardsonii* (claspingleaf pondweed)
PRO	*Potamogeton robbinsii* (Robins' pondweed)
PSP	*Potamogeton spirillus* (snailedseed pondweed)
PZS	*Potamogeton zosteriformis* (flatstem pondweed)
RAN	*Ranunculus* spp. (white water buttercup)
SPO	*Spirodela polyrhiza* (greater duckweed)
VAL	*Vallisneria americana* (wild celery)
UTV	*Utricularia vulgaris* (bladderwort)